

## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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| <b>(21) International Application Number:</b> PCT/US98/03962<br><br><b>(22) International Filing Date:</b> 2 March 1998 (02.03.98)<br><br><b>(30) Priority Data:</b><br><table border="0"> <tr> <td>08/808,325</td> <td>28 February 1997 (28.02.97)</td> <td>US</td> </tr> <tr> <td>08/808,327</td> <td>28 February 1997 (28.02.97)</td> <td>US</td> </tr> <tr> <td>08/808,733</td> <td>28 February 1997 (28.02.97)</td> <td>US</td> </tr> </table><br><b>(71) Applicant (for all designated States except US):</b> CEPHEID [US/US]; 1190 Borregas Avenue, Sunnyvale, CA 94089 (US).<br><br><b>(72) Inventors; and</b><br><b>(75) Inventors/Applicants (for US only):</b> CHANG, Ronald [US/US]; 3312 Hoover Street, Redwood City, CA 94063 (US). CHRISTEL, Lee, A. [US/US]; 3747 La Donna Avenue, Palo Alto, CA 94306 (US). KOVACS, Gregory, T., A. [US/US]; 105 Peter Coutts Circle, Stanford, CA 94305 (US). MCMILLAN, William, A. [US/US]; 8051 Presidio Drive, Cupertino, CA 95014 (US). NORTHROP, M., Allen [US/US]; 616 Vistamont Avenue, Berkeley, CA 94708 (US). PETERSEN, Kurt, E. [US/US]; 3655 Valley Ridge Lane, San Jose, CA 95148 (US). POURAHMADI, Farzad [US/US]; 41013 Pajaro Drive, Fremont, CA 94539 (US). YOUNG, Steven, J. [US/US]; 105 Milani Court, Los |                             | 08/808,325  | 28 February 1997 (28.02.97) | US | 08/808,327 | 28 February 1997 (28.02.97) | US | 08/808,733 | 28 February 1997 (28.02.97) | US | Gatos, CA 95030 (US). YUAN, Robert [US/US]; 2234 Semeria Avenue, Belmont, CA 94002 (US).<br><br><b>(74) Agents:</b> CHOW, Y., Ping et al.; Heller Ehrman White & McAuliffe, 525 University Avenue, Palo Alto, CA 94304-1900 (US).<br><br><b>(81) Designated States:</b> AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).<br><br><b>Published</b><br><i>Without international search report and to be republished upon receipt of that report.</i> |
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**(54) Title:** HEAT EXCHANGING, OPTICALLY INTERROGATED CHEMICAL REACTION ASSEMBLY

**(57) Abstract**

An assembly for performing controlled heat exchanging reactions is provided. The assembly has a chemical reaction chamber adapted to receive a sample and allow the sample to chemically react; a thermal sleeve having heating elements for making efficient thermal contact with a reaction chamber; an instrument with a housing comprising an electrical connection, a cooling source, a processing area adapted to receive a thermal sleeve, and an optics assembly in optical communication with a chemical reaction chamber; and a circuit for monitoring and controlling the optics assembly and for collecting the output signal of the optics assembly. Preferably there are a plurality of housings and associated modules, each of which may be independently controlled for heat exchanging operations.

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## HEAT EXCHANGING, OPTICALLY INTERROGATED

### CHEMICAL REACTION ASSEMBLY

#### FIELD OF THE INVENTION

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The present invention is a device useful for heating exchanging chemical processes and methods of manufacturing the device.

#### BACKGROUND OF THE INVENTION

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There are many applications in the chemical processing field in which it is desirable to precisely control temperature of chemicals and to induce rapid temperature transitions. In those reactions, heat is exchanged between chemicals and their environment to increase or decrease the temperature of the chemicals reacting. Thus, the term "heat exchanging" is herein intended to mean heat which may be transmitted by a heating source and absorbed by chemicals or heat released by the chemicals due to exposure to a cooling source. It is often desirable to control the temperature change in a manner which accurately attains the target temperature, avoiding under shooting or over shooting of the temperature and quickly reaches the target temperature. Such control of temperature may inhibit side reactions, the formation of unwanted bubbles, the degradation of components at certain temperatures, etc. which may occur at non-optimal temperatures. It is of further interest to be able to optically observe and monitor the chemical reaction.

Applications for heat exchanging chemical reactions may encompass organic, inorganic, biochemical and molecular reactions, and the like. In organic and inorganic reactions, chemicals may be heated to achieve the activation energy for the reaction. Examples of thermal chemical reactions include isothermal nucleic acid amplification, thermal cycling amplification, such as polymerase chain reaction

(PCR), ligase chain reaction (LCR), self-sustained sequence replication, enzyme kinetic studies, homogenous ligand binding assays, and more complex biochemical mechanistic studies that require complex temperature changes. Additionally, temperature mediated lysis of target microorganisms prior to gene amplification and  
5 detection can be accomplished by controlling temperatures and may be performed in the same reaction device as the amplification step. Temperature control systems also enable the study of certain physiologic process where a constant and accurate temperature is required.

Numerous devices and systems have been described in the art for conducting  
10 thermal transfer reactions. These devices use a variety of designs for heat transfer, such as water baths, air baths, and solid blocks such as aluminum. Chemical reactions in small reaction volumes have also been described.

Conventional instrumentation, for example, typically consists of a block of aluminum having as many as ninety-six conical reaction tubes. The aluminum block  
15 is heated and cooled either by a Peltier heating/cooling apparatus, or by a closed-loop liquid heating/cooling system, flowing through channels machined into the aluminum block. Because of the large thermal mass of the aluminum block, heating and cooling rates are limited to about 1°C/sec resulting in longer processing times. For example, in the PCR application, fifty cycles may require two or more hours to  
20 complete.

Part of the reason for the relatively large metal block is to provide enough mass to insure a constant and uniform temperature at each reaction site, as well as from site to site. Some heat transfer reaction instruments also incorporate a top-plate which is heated and cooled to ensure a uniform temperature across the top of all  
25 sample solutions. The sample inserts are tapered to maximize thermal contact between the insert and the metal block. One problem with this system is that the large thermal masses, required for temperature uniformity, take a long time (and or a

large heating/cooling power source) to heat and to cool. Usual heating and cooling rate for these types of instruments are on the order of 1-3°C/second.

Typically, the highest heating rate obtainable in commercial instruments is on the order of 3°C/second, and cooling rates are significantly less. With these  
5 relatively slow heating and cooling rates, it has been observed that some processes requiring high control of temperature are inefficient. For example, reactions may occur at the intermediate temperatures, creating unwanted and interfering side products, such as in PCR "primer-dimers" or anomalous amplicons, which are deleterious to the analytical process. The low control of temperature results in over  
10 consumption of reagents necessary for the intended the reaction.

Furthermore, for some diagnostic and environmental chemical detection methodologies, the volume of the tested unknown sample can be important. For example, in the detection of viruses in blood or other bodily fluids using PCR, the detection limit is about 10 virions. Therefore, a minimum fluid volume is required  
15 depending upon the concentration of virions in the sample. By way of illustration, at a concentration of 100 virions/mL, the sample size should be at least 0.1 mL; for more dilute samples, even larger sample volumes are necessary. Therefore, the chemical analysis system must be designed to handle a wide range of fluid volumes, from nanoliters to milliliters.

20 Another fundamental element of the many chemical reactions is monitoring of the chemical process and detection of the resulting product. Real-time monitoring of the process as it is occurring allows for accurate and quantitative data to determine the progress of the reaction and adjust the heating/cooling parameters accordingly. Where multiple cycles are conducted, the process may be monitored after each  
25 thermal cycle. After the reaction is complete, the product must be determined. In some processes, the product is separated prior to detection.

A preferred detection technique for the analysis is optical interrogation, typically using fluorescence, photosynthesis or chemiluminescence. For ligand-

binding assays, time-resolved fluorescence and fluorescence polarization are often used. Optimum optical sensitivity in fluid solutions can be attained by maximizing the optical sampling path-length of both the light beams exciting the chemical molecules and the emitted light that will be detected to generate the optical signal.

5           The control of heating and cooling changes may be referred to as thermal cycling. The term "thermal cycling" is herein intended to mean at least one change of temperature, *i.e.* increase or decrease of temperature, in the environment to which chemicals are exposed. Therefore, chemicals undergoing thermal cycling may shift from one temperature to another and then stabilize at that temperature, transition to a  
10 second temperature or return to the starting temperature. The temperature cycle may be performed once or repeated as many times as required by the particular chemical reaction. The various chemical reactions occurring during these temperature cycles are more specific and more efficient when the temperature is raised and lowered to the various required reaction temperatures as quickly as possible and controlled very  
15 precisely.

### Conventional Devices for Controlling Enzyme-Mediated Reactions

Devices which control the transfer of heat for chemical reactions are applicable for synthesis reactions such as thermal cycling PCR to amplify a segment of nucleic. In this methodology, a DNA template is used with a thermostable DNA  
20 polymerase, *e.g.*, Taq DNA polymerase, nucleoside triphosphates, and two oligonucleotides with different sequences, complementary to sequences that lie on opposite strands of the template DNA and which flank the segment of DNA that is to be amplified ("primers"). The reaction components are cycled between a higher temperature (*e.g.*, 95°C) for dehybridizing double stranded template DNA, followed  
25 by lower temperatures (40-60°C for annealing of primers and 70-75°C for polymerization). Repeated cycling among dehybridization, annealing and polymerization temperatures provides exponential amplification of the template

DNA. For example, up to 1  $\mu\text{g}$  of target DNA up to 2 kb in length can be obtained with 30-35 cycles of amplification from only  $10^{-6}$   $\mu\text{g}$  of starting DNA.

Polynucleotide amplification has been applied to the diagnosis of genetic disorders, the detection of nucleic acid sequences of pathogenic organisms in a variety of samples including blood, tissue, environmental, air borne, and the like; the genetic identification of a variety of samples including forensic, agricultural, veterinarian, and the like; the analysis of mutations in activated oncogenes, detection of contaminants in samples such as food; and in many other aspects of molecular biology. Polynucleotide amplification assays can be used in a wide range of applications such as the generation of specific sequences of cloned double-stranded DNA for use as probes, the generation of probes specific for uncloned genes by selective amplification of particular segments of cDNA, the generation of libraries of cDNA from small amounts of mRNA, the generation of large amounts of DNA for sequencing and the analysis of mutations. Instruments for performing automated PCR chain reactions via thermal cycling are commercially available.

Some of the instrumentation suitable for newer processes, requiring "real-time" optical analysis after each thermal cycle, has only recently become available. For example, the Perkin Elmer (PE) 7700 (ATC) instrument as well as the PE 9600 thermal cycler are based on a 96-well aluminum block format, as described *supra*, and thus are limited by slow heating and cooling steps and complicated optics. Optical fluorescence detection in the PE 7700 is accomplished by guiding an optical fiber to each of the ninety-six reaction sites. A central high power laser sequentially excites each reaction tube and captures the fluorescence signal through the optical fiber. Since all the reaction sites are sequentially excited by a single laser and fluorescence is detected by a single spectrometer and photomultiplier tube, complex beam-guiding and optical multiplexing are required.

An instrument from Idaho Technologies monitors each reaction tube in sequence as capillary sample carriers are rotated past heating and cooling sites and

optical interrogation sites. This instrument is much simpler than the ATC, however, it is not easily configured for commercial, high throughput PCR diagnostic applications.

A third real-time PCR analysis system is the MATCI device developed by Dr. Allen Northrup et al., as disclosed in U.S. Patent No. 5,589,136, incorporated herein by reference. This device uses a modular approach to PCR thermal cycling and optical analysis. Each reaction is performed in its own thermal sleeve and each sleeve has its own associated optical excitation source and fluorescence detector. Using a new generation of blue LED's, simple optics and solid-state detectors, real-time data can be obtained from a compact, low-power module. Not only are the optics simple and efficient, but the low thermal mass of the thermal cycling sleeve allows the MATCI device to realize extremely fast thermal heating and cooling rates, up to 30°C/sec heating and 5°C/sec cooling.

However, disadvantages to this MATCI device is in its use of a high thermal conductivity micromachined silicon holder, incorporating a heating element directly deposited on the silicon micromachined chip. Two drawbacks of this device are that the brittle silicon chip may crack and chip, and there is difficulty in micromachining a silicon chip such that a heater can precisely accept a tapered molded plastic insert for optimized thermal contact with the heating element itself.

For the reasons stated above, optimization of many biochemical reaction processes, including the PCR process, require that the desired reaction temperatures be reached as quickly as possible, spending minimal time at intermediate temperatures. Therefore, the heating and cooling system in which the sample reacts should be designed to optimize heating and cooling rates. It is also desirable that such a system is adapted to permit real time optical interrogation and to accept various sizes of samples.

## SUMMARY OF THE INVENTION



An assembly for controlling and performing heat exchanged chemical reactions is provided. The assembly is designed for optimal thermal transfer to or from a sample and efficient optical viewing of a chemical reaction with the sample.

5       The assembly has a heat exchanging chamber adapted to receive a sample. The chamber has a housing comprising: a rigid support; a reservoir which is adapted to receive the sample; and a thin flexible structure defining at least a portion of the reservoir and coupled to said rigid support. The flexible structure is conformable to the surface of an external heating element to contact the external heating element  
10       surface for optimal thermal conduction. A sealable port is present for introducing the sample into the reservoir.

      In another embodiment of the chemical chamber, two opposing major faces are joined by a plurality of contiguous minor faces to form an enclosed chamber in which the ratio of the thermal conductance of the major faces to that of the minor  
15       faces is at least about 2:1. Two of the minor faces are offset at an angle and are adapted to transmit light. The chamber includes a port for introducing a sample into said chamber and a seal.

      The assembly further includes a thermal sleeve having a slot for introducing a sample vessel, such as the heat exchanging vessel, into the sleeve, a heating element  
20       integrated with a wall of the sleeve, a contact surface on the wall for abutting with a portion of the sample vessel, where the vessel is present; and an electrical connection for applying current to the heating element.

      Further to the thermal sleeve, biasing elements may be present on wall of the sleeve to press the contract surface against a portion of the sample vessel. In one  
25       embodiment, the walls are opposing parallel plates and the sample vessel is placed between the plates.

Additionally, an instrument is for controlling the temperature changes of a sample and viewing the housing. The instrument has a housing comprising an electrical connection, a cooling source, a processing area adapted to receive a thermal sleeve, and an optics assembly in optical communication with the sleeve,  
5 where the sleeve is inserted into the assembly; and a circuit board for controlling the optics assembly and for collecting the output signal of the optics assembly.

Preferably there are a plurality of housings and associated modules positioned onto a support, each of which may be independently controlled for heating and/or cooling. A controller board monitors and controls the heat exchange and collects and  
10 analyzes data.

## BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows a partially exploded perspective view of a heat exchanging reaction chamber according to the present invention, wherein the reaction portion  
5 side walls are removed and the interior reaction portion is shown and wherein a sample port is unsealed by an attached cap.

Figures 2a, b show schematic perspective views of a heat exchanging module with a chamber associated with a thermal sleeve according to the present invention, wherein Figure 2a shows the module prior to biasing the sleeve against the inserted  
10 chamber and Figure 2b shows the module after the sleeve is made to bias against the chamber.

Figure 3 shows a top view of a reaction chamber inserted in a thermal sleeve with heating element and external cooling unit.

Figures 4a, b, c, d show various schematic perspective views of another  
15 chemical reaction chamber according to the present invention with major and minor faces angled to optimize optical viewing, wherein and Figure 4a shows a side view of the chamber, Figure 4b shows a front view of the external surfaces of the chamber, Figure 4c shows a front view of the interior of a chemical reaction chamber with a sample channel leading to the reservoir, Figure 4d shows a top view of the chamber.

20 Figure 5 shows a perspective side view of a reaction chamber with integral lenses for optical detection and in associated arrangement with an external light emitting source and detector.

Figure 6 shows a perspective view of a thermal sleeve with one heating plate attached to a support and for illustration purposes the other plate is removed from the  
25 support.

Figure 7 shows a perspective view of another heat exchanging assembly with a chamber inserted in a thermal sleeve.

Figure 8a, b, c, d show various heating and cooling configurations of a thermal sleeve according to the present invention, wherein Figure 8a is a top view of the heating and cooling elements of the sleeve, Figure 8b is a front view of the cooling elements shown in Figure 8a, Figure 8c is a front view of another sleeve with heating and cooling elements and Figure 8d is a side view of the sleeve shown in Figure 8c.

Figure 9 shows a perspective view of a controlled heat exchanging instrument with thermal sleeve, optics assembly coupled to circuit boards and cooling unit and a reaction vessel exploded from the instrument.

Figure 10a, b show perspective views of a heat exchange assembly according to the present invention including instrument with optics assembly and cooling unit, wherein Figure 10a shows a front view of such an assembly and Figure 10b shows a side view of such an assembly.

Figure 11a, b and c show a perspective view of a cluster of heat exchanging assembly units on a base support according to the present invention, wherein Figure 11a shows four units interfacing with a main controller board, Figure 11b shows one of the units in Figure 11a in pneumatic and electrical communication with a base support and Figure 11c shows eight units each interfacing individual and separate controller boards.

## DETAILED DESCRIPTION OF THE INVENTION

In general, this invention provides a low thermal mass mechanical heating and cooling assembly which is adapted for rapid heating and/or cooling of chemical solutions to perform reactions and for efficient detection of the reaction products. The low thermal mass of the system ensures rapid heating and cooling since there is little material to heat and cool and because there is a high surface to volume ratio for

thermal transfer. Those skilled in the art have only recently appreciated that rapid cooling and heating improves efficiency and decreases the amount of extraneous undesirable reactions, and that certain reactions, could be performed with high thermal exchange rates. However, no device has been capable of efficient and independent thermal exchange as well as detection in each of the device units until the current invention.

The objectives of the invention are to greatly increase thermal exchange rates in chemical processes, such as PCR (up to 10X faster heating and cooling rates), to optimize temperature uniformity of the reactants, to accommodate high thermal expansion coefficients to minimize thermal-induced mechanical stress; maximize optical excitation efficiency (long optical pathlength), to maximize optical detection sensitivity (maximize photon collection volume), to maximize fault detection capability, to minimize computer overhead of the host instrument, and to minimize overall power consumption of the instrument via independent, modular, intelligent reaction chambers supported by a powerful instrument platform and technology for long term versatility.

The objectives of the invention are attained via a self-contained cooling and heating reaction assembly with optics. Each controlled heat exchanging assembly constitutes a single reaction site. Overall, the assembly unit comprises (a) a reaction chamber which is compatible with a heating element; (b) a thermal sleeve having integral heater or cooling elements wherein a portion of the reaction chamber surface makes intimate contact with a surface of the sleeve; and (c) an instrument for housing the sleeve having an optical assembly incorporating solid-state excitation and detection sources and a cooling system. The instrument also has one or multiple circuit boards with microcontrollers for monitoring control of light excitation source and detection source and communicate with a host. The thermal exchange assembly may additionally have a support with a main controller board which communicates with the instrument, sleeve and reaction chamber to control procedures such as for temperature and optical control wherein each instrument may be separately controlled

or the cluster of instruments may be under a single set of controls; conduct self-diagnostic protocols; and receipt, storage and processing of data.

### Heat Exchanging Chamber

Figure 1 shows the interior of a heat exchanging reaction chamber according to the present invention which is adapted to receive a sample for chemical reacting. The chamber is designed for optimal thermal conductance and efficient optical viewing of the sample. The thin shape contributes to optimal thermal kinetics by providing large surfaces for thermal conduction and surfaces are adapted to make intimate contact with temperature regulating elements. In addition, the minor or major walls may provide windows into the chamber such that the entire reaction sample can be optically interrogated. In addition, the chamber is suitable for a wide range of reaction volumes.

In more detail to the components shown in exemplary embodiment in Figure 1, a chemical chamber (2) has a port (4) which is in fluidic communication with a housing (6) having channel (8) connecting the port to a reservoir (10). A seal cap (12) is attached to housing (6) by a flexible arm (14) and is adapted to cover the port (4) sealing it close. The cap (12) is insertable into the port (4) to engage channel (8). A rigid support frame (16) and thin flexible walls (18) shown in Figure 1 exploded from the chamber, both define the reservoir (10), wherein the flexible walls are coupled to the frame. On the rigid support are reflective faces (20) which bounce back light transmitted from the reservoir allowing for increased detection of signal.

In using the chamber, a sample added to port (4) flows through channel (8) and into reservoir (10). In the reservoir (10) the sample is introduced to chemicals for reacting. The walls of the reservoir is made to press against a heating or cooling element. Preferably, the walls conform to the element surface. The sample is exposed to variations in temperature by activating the heating/cooling element. The reaction and/ or reaction products are optically viewed.

The thin flexible structure (18) which defines at least a portion of the reservoir, facilitates optimal thermal conductance to the chemicals contained in the reservoir. The flexible nature of the walls (18) allow for maximum contact with a heating or cooling source. The structure is typically conformable to the surface of an external thermal element such that the surface of the flexible structure may adapt to the shape of the external heating/cooling element surface in a manner that avoids or minimizes gaps between the two surfaces. Furthermore, the flexible wall continues to conform to the thermal surface as the surface shape may change during the course of the heat exchanging operation. For example, as the heating element expands due to the increased temperature, the chamber wall will also expand to maintain optimal contact with the heating element. Also, if the walls expand due to an increase of internal pressure within the reservoir, the walls do not become rigid, but remain conformed to the heating or cooling surface. Contact may be made by biasing the thermal source against the walls and/or by pressing the walls against the thermal surface.

Figures 2a and 2b demonstrate the contact one chemical chamber makes with a thermal sleeve to form a heat exchanging module (30). In Figure 2a, thermal sleeve (32) with heating or cooling plates (36), (38) are in a relaxed position with opening (34) between the plates. However, as depicted in Figure 2b, when chamber (46) with flexible walls (48) is inserted in the opening between two angled heating or cooling plates (36), (38), the plate surfaces (40), (42) fully engage the chamber walls (48). In this activated position, minimal or no gaps are found between the plate surfaces and chamber surfaces. The plates (36), (38) are made to bias against the chamber walls (48) by springs (44). In the alternative, the chamber is made to press against the thermal plates. The conformable chamber walls mold to the angled shape of the heating surface to provide maximum thermal contact between surfaces.

Further to the ability of the chamber to optimize thermal kinetics, the flexible wall is of low thermal mass to permit rapid temperature transfer. The thin walls of the chamber also promote transfer of both hot and cool temperatures to the reservoir.

In Figure 3, the top view of a reaction chamber (50) is shown to be in intimate contact with heating elements (52) and surrounded by cooling chamber (54). The thickness of the wall structure is between about .0001 to .020 inch, typically about .0005 to .005 inch and preferably about .001 to .003 inch. In order to achieve this  
5 thinness, the wall may be a film, sheet, or a molded, machined extruded or cast piece, or other convenient thin and flexible structures.

The material composing the wall may be a polyalcohol including polypropylene, polyethylene, polyester, and other polymers, laminates or homogenous polymers, metals or metal laminates, or other materials which may be  
10 thin, flexible, conformable and permit high heat transfer and preferably in the form of a film or sheet. Where the frame of the chamber which supports the walls is a particular material, such as polypropylene, the walls are preferably the same materials, such as polypropylene, so that the heat expansion and cooling rates of the walls are the same as the frame. Therefore, undue heat or cooling-induced stresses on  
15 the materials is minimized such that the sleeve walls maintain the same contact with the frame and wrinkling of the walls is avoided during multiple temperature cycling.

Although the flexible walls are preferred, in some embodiments, the walls which contact the heating elements may also be rigid and flat to communicate with a rigid and flat heater. Whether rigid or flexible, the walls typically make up the major  
20 faces of the chamber to allow for maximum heat or cooling exposure to the chemicals contained therein. The chamber also has at least one minor face to a plurality of minor faces which provide a frame or support for the major faces.

The major and minor faces of one sealed chamber is shown in the variously throughout Figures 4a, b, c, d with major and minor faces angled to optimize optical  
25 viewing. A plurality of minor faces, two of which are shown in the Figure 4a, minor faces (62), (64) couple two opposing major faces (72), (74) (only one major face is shown) together to form the chamber (60). Illustrated in Figure 4b, a major face (74) may be surrounded by five contiguous of minor faces (62), (64), (66), (68), (70).



In Figure 4b, angled minor faces (64), (66) are coupled together to form an angle on one end and to parallel minor faces (62), (68) on the other. The angled faces (64), (66) may define the bottom portion of the reservoir (76), and a back walls (78) may define the top portion of the reservoir (76) as depicted in the interior view of one chamber in Figure 4c wherein a sample channel (80) leads to the reservoir (76). The channel and reservoir including the backwalls is optionally an separate piece inserted into the main body of the chamber. The channel leading to the reservoir optionally serves a variety of functions including providing a conduit for filling the reservoir, such as bottom filling or an area to hold overfilled reagents and purged air.

10 The angled faces (64), (66) may be joined to form a "V" shaped point (82) (triangular), especially on the bottom of the chamber to allow for easier filling by reducing or eliminating bubble formation. Alternatively, the interface of the angled faces need not connect form a point but is separated by an intermediary portion, such as another minor face or various mechanical or fluidic features which do not significantly interfere with the thermal and optical performance of the chamber. For example, the angled faces may meet at a port which leads to another processing area in communication with the reaction chamber, such as an integrated capillary electrophoresis area.

20 The top minor face (70) is coupled to the ends of the parallel faces (62), (68) in one embodiment shown from the top view in Figure 4d. The top face (70) is also in contact with a port (not shown) for adding liquids and removing air. The port allows access of a pipette tip through a channel from the outside into the interior of the chamber, for example, to the bottom of the reservoir to enable bottom-up filling. The port may also permit other conventional methods of sample introduction, such as through an automated fluid injection system or through a fluidic manifold which optionally is an integral part of the chamber. The chamber may also be one aspect of a larger device which processes the fluid prior to the fluid flowing through the port and into the chamber. One example of a disposable fluidic cartridge is in copending

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patent application, U.S.S.N. 08/998,188, filed December 24, 1997, the contents of which is herein incorporated.

The external terminus of the port is designed to be sealed, preferably by accepting a seal cap (84), wherein one embodiment is also shown in Figure 4d. The cap (84) provides a means for sealing the port after filling to provide a barrier between the thermally controlled interior reaction volume and the non-thermally controlled environment to inhibit contamination of the sample, to prevent evaporation of fluid during heating of the sample, and the like. In various embodiments anticipated by the present invention, the seal may be a snap-on cap, a screwtop, or other specialized closure as needed for the selected analytical protocol. Such a cap may be composed of any convenient material such as polypropylene or an elastomer, such as Santoprene<sup>TM</sup> (trademark of Monsanto Corporation, located in San Louis. Missouri). In one embodiment, the chamber may be further sealed from the exterior environment by the heating of plastic material on or composing the top minor face. In another embodiment the seal is created by a drop of oil placed on top of an aqueous sample to prevent evaporation of the sample. A cap may also be a plug which is inserted into a channel in communication with the reservoir such that the plug creates an increase in pressure within the reservoir. The increased pressure may allow for a solution in the reservoir to remain in a liquid state without going into a gaseous state at certain high temperatures. This property is in accordance with the theoretical principle,  $PV=nRT$ , where P is pressure, V is volume, n is moles, R is a constant and T is temperature. The resulting increased pressure may also cause outward expansion of the flexible walls to force the walls against the external heating units creating a better contact between the walls and the heating elements.

The chamber may be configured to optimize the visualization of the reaction in the reservoir. To this end one, two or multiple, and typically two minor faces comprise the optical windows. Where two windows are present, one window serves as a light excitation entry port and the second for emitted light detection. In a preferred embodiment, both windows serve for excitation and detection for two light

paths wherein one light path light is radiated through one window and detected through the second window and in the second light path light is emitted through second window and detected through the first window. The window faces may be offset at an angle selected to maximize the detection process.

5 One chamber (90) associated with an external optics assembly (92) is shown in Figure 5. Associated optical system (92) is designed to illuminate one minor optical faces (94), (96) with optical excitation radiation by individual light source (98) and to detect light emitted from the reservoir contents and through the adjacent minor optical face with detector (102). In the alternative, both adjacent minor faces  
10 (94), (96) may receive radiation from a light source (98), (100) and observation by detectors (102), (104) is at the other respective adjacent face, where the excitation light which is radiated through each face is a different wavelength and light which is detected at each respective adjacent face is also a different wavelength. Exemplary paths of differing wavelengths of excitation and detection light are shown by arrows  
15 in Figure 5. The faces (94), (96) additionally have two lens (106) directly molded into the surfaces to direct the light.

Where excitation and detection occurs at different faces as in Figure 5, it is usually preferred that the optical faces are offset at an angle (A). The preferred angle is about 90°. A 90° angle between excitation and detection optical paths assures that  
20 a minimum amount of excitation radiation entering through one minor face will exist through the other detection minor face. Also the 90° permits a maximum amount of emitted radiation, *e.g.* fluorescence, to be collected through the detection window. In other embodiments, the angle between adjacent optical faces is larger or smaller than 90°, depending, *inter alia*, on the efficiency and sensitivity of the excitation and  
25 detection optics. For example, where a detection system effectively discriminates between excitation and emitted light, an angle of less than 90° between faces may be desired. Conversely, where a detection system fails to efficiently discriminate between excitation and emitted light, an angle greater than 90° may be of interest.

One to a plurality of light transmissive elements may be present on the angle forming minor faces. The optical elements may be designed, for example, to maximize the total volume of solution which is illuminated by an LED fluorescence excitation source, to focus an optical excitation source on a specific region of the reaction chamber, or to collect as much fluorescence signal from as large a fraction of the reaction chamber volume as possible. In addition, gratings for selecting specific wavelengths, filters for allowing only certain wavelengths to pass, and multiple lenses or filters optimized for multiple excitation sources or detectors may be used. In another embodiment, the opposite face may be optimized to collect and focus the maximum percentage of emitted fluorescence signal from the solution to an array of photodetectors. Alternatively, the faces may be simple, clear, flat windows serving as optically transmissive windows. Other elements include colored lenses to provide filtering functions, retro-reflective surfaces, optical grating surfaces, etc.

Further to the reaction chamber, the major or minor faces may be adapted for additional optical interrogation. The surface may be coated or comprise materials such as liquid crystal for augmenting the absorption of certain wavelengths. The surfaces may be used to determine the temperature of the enclosed chemicals by detecting particular absorption bands which reflect temperature conditions.

Thin films of metals, polymers, and combinations of materials such as in laminates, not only can be employed in a reaction chamber for the structural and thermal properties, but for optical properties, as well. Thin films constitute material thickness ranging from a few angstroms to hundreds of microns in thickness, and are usually formed with a particular series of processes familiar to those in the art of vapor deposition, plasma deposition, magnetron and RF sputtering, laser ablation, etc.... For example, vapor-deposited thin films of silver can augment the detection and collection of raman (inelastic scattering of an optically excited source) spectra. This and other materials can be deposited on a variety of substrates (glass, plastic, silicon, metals, etc...) to be translucent (transmitting) in certain wavelengths at angles of incidence, and reflective in others. This is the basis of a lot of optical materials

developments and devices such as dichroic beam splitters, dielectric band pass filters, neutral density filters, etc....

The use of these capabilities to manufacture films that can be attached to, used to hermetically seal reaction tubes, or are deposited directly onto the wall of a reaction tube that are being interrogated optically, can result in reaction tubes with specific optical emission and excitation properties. These thin films processes when used economically, can thereby be used to manufacture reaction tubes or vessels inexpensively resulting in disposable, tubes with fine-tuned optical properties.

The chamber may be fabricated in various ways. It may be molded in several pieces which are bonded together or injection molded in one piece. There are several advantages to the multi-piece design and manufacturing approach. One benefit is that very thin walls can be achieved where the walls can be consistently produced to be the same size and shape. Another benefit is that the optical features of the device are separated from the fluidic features such that both components can be independently designed and optimized. For example retro-reflective walls may be made on one or many sides of the reservoir to reflect light. A third advantage is that the primary optical component can be fabricated from a different material than the primary fluidic component. An additional benefit is that the major surfaces may be fabricated from a different material than some or all of the minor surfaces. For example, materials with optimal thermal characteristics may be different from those with optimal optical characteristics. In particular, the angled optical windows, with or without light components, could be molded from polycarbonate, which has good optical transparency, while the rest of the chamber could be molded from polypropylene, which is inexpensive and is known to be compatible with the sensitive PCR reaction. Both pieces can be bonded together in a secondary step. The optical window is press-fitted or bonded into an end of the chamber, preferably the bottom of the chamber.

In one method of fabricating a chamber where the major and minor faces are different materials, the minor faces of the frame is molded with a reservoir having open sides. The chamber frame is made by standard injection molding processes for thermal materials. After the frame is made, the major walls are produced by placing, and preferably stretching, the material over the reservoir area. The walls are then bonded to the frame. Where the walls are a film or sheet, the material may be attached by heat sealing, adhesive bonding, ultrasonic bonding, etc. over the reservoir of the chamber.

A chamber in which the major and minor faces are fabricated from the same material requires that the total surface area of the major surfaces be at least about twice that of the total surface area of the minor surfaces where thermal conductance ratio of 2:1 is desired. On the other hand, if the faces are made of different materials, it is possible to modify the geometry from that shown since major faces comprised of materials with high thermal conduction could be combined with minor faces of low thermal conduction. The faces may be fabricated from glass or polymers including polyacrylics, polyamides, polycarbonates, polyesters, and vinyl polymers, or any combination thereof. Polycarbonates are preferred for the optically transparent faces.

An insert separate from the main frame of the chamber may be placed inside of the chamber to define some of the reservoir or other internal features. The insert may fill the top of the chamber and provide some of the faces. The insert may be bonded or preferably press-fitted into the chamber. The insert may also provide the channel, port, and cap attachment means.

The shape of the chamber may differ according to the particular reaction being performed and the associated thermal transfer device. Furthermore, the relationship of the frame to the flexible walls may vary as long as the frame is coupled to the walls and the walls are accessible to contact and external thermal source. For example, the minor faces may surround the perimeter of the major faces, as describe above in Figures 4a-d, In the alternative, the minor faces may provide a

box frame which supports a centrally suspended pouch which is comprised of the flexible major faces. The chamber may be sized, in particular in the reservoir region of the chamber, to contain volumes from nanoliters to milliliters, depending upon the desired use. Preferably, the volume of the chamber is from about 1 to about 1000  
5 microliters, and typically from about 1 to about 500 microliters, and more preferably from about 10 to about 100 microliters.

In summary of the reaction chamber, the various embodiments of the chamber have the following characteristics: high surface-to-volume ratio for efficient heating/cooling; thin, low-mass walls; conformable walls to maximize association  
10 with heating and cooling system; moldable and bondable material where multiple pieces are required; features which accommodate high thermal expansion coefficients to minimize temperature-induced mechanical stress during heat exchanging operations; chemically inert materials so that there is no adsorption or reaction with reactants, intermediates, or products, no or minimal inactivation of  
15 enzymes by surface active means, and compatible with glycerol; windows with high optical clarity for efficient interrogation; long excitation optical path lengths; maximized offset between excitation and emission detection windows; no or minimal light coupling between excitation and detection devices; optical elements such as retro-reflective surfaces; major walls precisely mated with module heating/cooling  
20 surfaces; port for introducing sample and reagents; means to preclude or minimize refluxing during cycling; efficient removal of air during filling and capping; and sealing of the reaction mixture from external environment.

### Thermal Sleeve

The chamber is compatible with a thermal sleeve for heating or cooling the  
25 chamber reservoir. The thermal sleeve is designed to induce a temperature change in the chamber by making intimate contact with the chamber.

Figure 6 shows a perspective view of one thermal sleeve (200) with one heating or cooling plate (202) attached to a support bridge (206) and another plate

(204) removed from the support (206). Each heating or cooling plates (202), (204) have one face being a contact surface (208) and another face being a biasing surface (210) with one end (212) of each plate being slanted towards each other. Further on each the biasing surface (210) is a spring (214) with an integral attachment arm (216) for coupling to support bridge (206) at securing region (218). The plate is partially inserted through bridge slots (220) and the plate attachment arm (216) is fastened to the bridge securing region (218). The biasing surfaces (210) of the plates also have a plurality of electrical connections (220) which may communicate with heating, cooling or electrical sources (not shown) or any combination thereof. When assembled, the plates are held in opposition by the support bridge to form an opening between the plates for enclosing a reaction chamber.

Figure 7 demonstrates how a chamber (252) may be enclosed within one embodiment of the thermal sleeve (200) depicted in Figure 6, to form a heat exchanging module (250). The contact surface (208) of each plate (202), (204) are made to press against the chamber surfaces (not shown) in a manner that maximizes thermal contact. In general, the sleeve may consist of one or more, and preferably two, separate spring-loaded heater plates configured to mechanically bias against the surface, *e.g.* reservoir area, of the reaction chamber. Such a spring-loaded configuration would simplify mechanical tolerances between the various mechanical components, *i.e.* thermal sleeve, chamber and optical unit. Alternatively, the plates may be made to bias against a reaction chamber surface by mechanical force initiated by other parts or a mechanical motor, thermal expansion, pneumatic pressure, such as air flow pressure, hydraulic pressure, and the like. The interior of the heat exchanging sleeve may also be tapered to allow for a snug fit with the reaction chamber insert. Furthermore, the walls of the inserted chamber may be expanded to also press against the heater plate surface.

The shape of the sleeve is designed for optimal thermal contact with a reaction chamber. In one embodiment, the opening in the sleeve for contacting the chamber surface is elongated in the x-direction. Thus the opening is longer in the



direction perpendicular to the length of a chamber. Preferably the shape of the opening is rectangular in cross section. The ratio of length to width of the surfaces defining the opening may be at least 2:1. Such elongation provides greater contact with an inserted chamber than prior designs where the opening for inserting a chamber is expanded in the z-direction and are typically round or octangular in shape to hold round tubes.

As described in this application, the achievement of rapid heat exchange (heating and/or cooling) from a sample in a reaction tube requires a low thermal-mass thermal sleeve assembly together with a thin, wide reaction tube. The fastest thermal cycling instruments to date (for example, the Light Cycler by Idaho Technologies and the MATCI by Lawrence Livermore National Labs) solve this problem by making the reaction tube a thin long cylinder, 1 mm in diameter or less. Other very fast thermal cycling instruments depend on very small liquid sample volumes, which are relatively easy to heat and cool very rapidly. However, these approaches are only suitable for very small liquid sample volumes (both the Light Cycler and the MATCI are designed for only 10-20  $\mu\text{L}$ ). In contrast, the tube described here is thin and wide. Instead of increasing the volume capacity by simply making the reaction tube longer, this application teaches that large heating and cooling rates can also be achieved by properly designed reaction tubes which are, instead, thin and wide. The counterpart and complementary design of the low-mass thermal sleeve assures that the entire assembly, including the relatively large liquid sample volumes (up to and over 100  $\mu\text{L}$ ), can be controllably heated and cooled at the maximum rates.

The thermal plates may be made of various materials. In order to ensure that the inside of the heat exchanging sleeve is resistant to bleach and other cleaning solutions, the interior may be coated or lined with a chemically inert material, such as a polytetrafluoroethylene, or the entire sleeve may be fabricated from a chemically stable material, such as a ceramic or metals such as aluminum nitride, aluminum

oxide, beryllium oxide, and silicon nitride. Other materials which may be utilized include, e.g., gallium arsenide, silicon, silicon nitride, silicon dioxide, quartz, glass, diamond, polyacrylics, polyamides, polycarbonates, polyesters, polyimides, vinyl polymers, and halogenated vinyl polymers, such as polytetrafluoroethylenes. Other possible materials include thermocouple materials such as chrome/aluminum, superalloys, zircaloy, aluminum, steel, gold, silver, copper, tungsten, molybdenum, tantalum, brass, sapphire, or any of the numerous ceramics, metals, and synthetic polymeric materials available in the art. A ceramic inside surface is preferred because the surface may be conveniently machined to very high smoothness for high wear resistance, high chemical resistance, and good thermal contact to reaction chamber inserts. Ceramic chambers can also be made very thin (between 0.025-0.050 mm) for low thermal mass. A heat exchanging chamber made from aluminum or copper also has high thermal conduction, but a larger thermal mass.

The heating source, such as heating resistors, may be directly screen printed onto a plate, particularly plates consisting of ceramic insulating materials, such as aluminum nitride and aluminum oxide. Screen printing provides high reliability and low cross-section for efficient transfer of heat into the chamber itself. In a preferred embodiment, the heating source is integral with the plates by baking the element inside of the ceramic unit. Also, thick or thin film resistors of varying geometric patterns may be integrated into the thermal jacket walls to provide more uniform heating, for example by having thicker resistors at the extremities and thinner resistors in the middle. Heating elements may consist of carbide, tungsten, silver, or other materials which heat when a voltage is applied to the material. One way of heating a metal sleeve is by using a laminated heater source such as an etched-foil heating element (Minco Products, located in Minneapolis, MN) attached to the surface of the heating plates. Optionally, cooling fins, of the same or different material as the body of the chamber, may be brazed, soldered, or epoxied directly over the screen-printed resistors.

The function of the support bridge, shown in the embodiment in Figure 6 and described above, is to serve as a support for one or more thermal sleeve heating or cooling elements and to provide a guide for inserting the reaction chamber into the main body of the thermal sleeve. The support may include a slot for inserting the  
5 chamber between thermal plates. The slot may incorporate mechanical features or a sealing surface which allow a tight mechanical seal.

A consideration in the choice of material for the top support is that its thermal expansion coefficient (TCE) match that of the thermal plate as closely as possible. The materials of construction recited above for the plate are also useful for the  
10 support. Appropriate combinations will be apparent to the skilled artisan. Where the thermal plates are made of ceramic, the preferred material for the top is also ceramic. If the plate is aluminum, the support will likewise be aluminum.

The mechanical transition between the thermal sleeve plate and top support is a critical joint. The heating or cooling plate may be cycled many times (up to  
15 400,000 over a 5 year life) e.g. in PCR applications between about room temperature, 60°C and 95°C, while the top support may be maintained at a relatively constant temperature. Thermal gradients and stresses are high in this region. Flexible, chemical-resistant adhesives and gasket materials may be used to ensure bonding. Preferred adhesives are epoxy, but a more robust metal sealing technique  
20 can be used if the thermal plate is metal or ceramic.

Another criteria for the transition region is that the sealing or bonding material and the method for joining the top support to the thermal plate should be resistant to bleaches and other cleaning solutions. It is expected that up to 1000 exposures to cleaning solutions such as 10% bleach and 1% Tween 20 may occur.

25 The thermal sleeve of this invention has high thermal conduction and low thermal mass to permit rapid heating and cooling. Further, the thermal sleeve is sufficiently durable for repetitive use (as many as 10,000 reaction chamber insertions). The heating elements are integrated into the sleeve to assure rapid,

efficient heating. To maximize cooling efficiency, cooling element may also be attached to the surface such as cooling fins or thermally conductive elements connected to a secondary cooling source. For example, the sleeve may be thermally connected to a Peltier element or to a heat pipe.

5           Figure 8a, b, c, d illustrates exemplary variations of heating and cooling configurations of a thermal sleeve. Figure 8a is a top view which looks directly down into the mouth (262) of the sleeve (260). The sleeve is provided with cooling fins (264) and integrated heaters (266). In this embodiment the sleeve is provided with a thin interior liner (268). Figure 8b is a front view of the cooling fins (264) shown in  
10   Figure 8a. Figure 8c is a front view of another sleeve (270) with heating (276) and cooling elements (274). A proportional-to-absolute zero (PTAT) temperature sensor is shown at (272). Figure 8d is a side view of the sleeve (270) shown in Figure 8c. In the side views of Figure 8d, screen printed or laminated heating elements (276) are shown beneath the cooling fins (274).

15           The temperatures of an inserted chamber and/ or thermal plates may be monitored by one or more sensor located on the thermal sleeve. In order to achieve the desired 0.5-1.0 °C temperature accuracy, silicon-based, proportional-to-absolute zero (PTAT) thermal sensors may be used. The output of the sensors is linearly proportional to temperature. High precision PTAT temperature sensors can be very  
20   small, e.g. 0.5x0.5x1.0 mm. Alternatively, thermistors, thermocouplers and resistance temperature detectors (RTD), especially RTD's made from platinum, copper, nickel and nickel-iron may be used. These sensors are easily affixed to the trailing edge of the heat exchanging reaction chamber.

          The thermal sleeve is also adapted for optical interrogation of the contents *in*  
25   *situ* by the provision of optically transmissive elements on the sleeve and may incorporate various features, such as lenses and filters, to facilitate optical visualization. In one embodiment, at least two surfaces of the sleeve are optically transmissive, preferably forming the bottom of the sleeve adjacent to the optical

windows of an inserted reaction chamber. An important criteria for the window material is transparency or translucency of the window. The window may also simply be an opening in the sleeve through which the chamber may be viewed. In one embodiment, the jacket is open at the bottom so that a portion of an inserted chamber may extend below the sleeve for direct optical interrogation. Where the window is a particular material, it is preferred that the window be as close a match as possible between the coefficients of thermal expansion (TCE) of the sleeve and the window. For example, a glass having a TCE closely matches that of a ceramic sleeve may be chosen. A plastic window is more suitable if the thermal sleeve is metal. It is also critical that the window material be stable to bleach and other cleaning solutions. In particular, it is envisioned that the inside of the thermal jacket will be periodically cleaned by pipetting in dilute bleach solutions, followed by water, followed by an alcohol dry. These solutions will be in direct contact with the joint between the optical window and the heat exchanger. This transition also significantly affects illumination and light-gathering functions of the device.

The mechanical transition between window and thermal elements is a critical joint. It is desirable to maintain the optics package at a relatively low temperature, or at least constant temperature, while the thermal sleeve is temperature cycled many times. Thermal gradients and stresses are high in this region. Another criteria for the transition region is that whatever sealing or bonding material and method for joining the optical window to the thermal sleeve is used should be resistant to bleaches and other cleaning solutions. In particular, it is envisioned that the inside of the thermal sleeve will be periodically cleaned by pipetting in dilute bleach solutions, followed by water, followed by an alcohol dry. These solutions will be in direct contact with the joint between the optical window and the thermal sleeve. This transition also significantly effects illumination and light-gathering functions of the device.

### Controlled Heat Exchanging Instrument

The optics assembly may be fabricated in an instrument which is configured to accept a thermal sleeve. Such an instrument may additionally have systems for maintaining the environmental temperatures, such as a cooling system, and various control mechanisms to regulate the operations being performed within the heat exchanging chamber.

In Figure 9, the instrument (600) is shown with housing (602) and associated operational elements. A processing area (604) is adapted to accept a thermal sleeve (630) and heat exchanging chamber (632) described *supra*. The processing area (604) is in pneumatic communication with cooling fan (606) by inlet channel (608) and with outlet channel (610) leading from the processing area to an outlet port (612). When a chamber (632) is inserted into processing area (604), the chamber reservoir is exposed to the cooling air circulating from fan to inlet channel (608), to processing area (604). Thereafter, the air travels through outlet channel (610) to exit the housing at port (612). In addition, an inserted chamber is in optical communication with optics assembly (620) including optics emission blocks and optics detection blocks coupled to circuit boards (622) for controlling the optics. The instrument also includes sensor (614) to monitor optics, heating or cooling operations.

The optical assembly consists of lensing elements, such as light collimating, *i.e.* light-pipe, and focussing elements, with transmissive and reflective filters, gratings, photodiodes, fresnel lens and the like, as needed, which may be attached to a circuit board which may contain LEDs and photodetectors. The lensing components may be injection molded from a transparent plastic such as polycarbonate or other moldable, optically clear plastic or glass. The lensing elements connect the reaction chamber windows to the excitation and detection optical components, as described below. The lensing elements incorporate and interface with filters and with the optical excitation and detection circuit board which contains the solid-state LEDs and photodetectors.

Solid-state LED's and photodetectors are optionally assembled onto a small circuit board located below the lensing components. This is a simple board, fitted with alignment features to accurately position the excitation sources and detectors with respect to the lensing elements and the reaction tube. An edge-connector or  
5 flex-connector provides electrical connection between the optical board and the adjacent controller board.

The housing may be machined from aluminum and anodized, molded from a rigid, high-performance plastic, or other conventional techniques and materials. The primary functions of the housing are to provide a frame for holding the thermal  
10 sleeve and top support, and optical assembly and to provide flow channels and ports for directing cooling fluid, *e.g.* air and efficiently guiding the fluid flow across the surface of the thermal sleeve/chamber.

A cooling source, such as gas diffusing plates or other air flow distributing structures for assuring uniform air flow around the thermal sleeve, a fan for blowing  
15 cool air over the sleeve, a Peltier device, liquid cooling such as water or a compressed gas cooling source, or the like. The cooling element may be directly formed in the housing or be fabricated independently from the housing and assembled into the housing at a later time. For example, each thermal sleeve in an array of heat exchanging assemblies may communicate with a cooling element. In  
20 addition, ports located in the housing may provide coolant air inlet and outlet ports. The ports may also be sealed to a base-plate using suitable sealants where the ports interface with inlet and outlet ports in base plate.

Further to the instrument, an alternative embodiment is shown in Figures 10a and 10b. The instrument (650) has thermal sleeve (670) (shown partially exploded  
25 from the instrument) with sleeve plates (672), which are attached to a top support (674) for mating with the instrument housing (652). Housing (652) has air inlet and outlet port (654), a support (656) with air diffusing plates (658) and an optics module

(660) with attached circuit board (662). The direction of cooling air flow is shown by the arrows.

The entire electronic control of each heat exchanging assembly may be incorporated into one or two circuit boards or chips attached to the sides of the housing. In the cross-sectional view of the instrument shown in Figure 10b, the thermal sleeve (670) with top support (674) is shown partially exploded out of the housing (652) having optics module (660), and optics circuit board (662) which interfaces with a pair of controller boards (664). The circuit board (662) and controller boards (664) may be fabricated using high reliability, low-profile surface-mount technology. The controller board (664) communicate with the optical board through a slot in the housing and a 90° electrical connector where the bottom end of the circuit board plugs into an electrical socket on the base-plate for electrical communication to the controller board.

Moreover, multiple assembly units may be grouped together, as in conventional reaction apparatus, such as PCR, for exposing the multiple samples to the same temperature profile, in which case only one unit need be equipped with electrical control circuitry. However, when it is desired to react multiple samples using independent conditions, then independent control of each unit or grouping of unit is needed.

In Figures 11a and 11b, a cluster of heat exchanging assembly units are arranged in an array. Figure 11a shows one embodiment a heat exchanging system (700) with four assembly units (710), one of which are shown with a side panel removed to expose the internal unit on base support (702). Figure 11c shows another embodiment of a heat exchanging system (800) with eight units (810) arranged on base support (802). In Figure 11a and in the cross-sectional view shown in Figure 11b, each unit has optics circuit board (712) which interfaces with the "mother" command unit (704), *e.g.* a common controller board. The single controller board has several, *e.g.* four, circuit boards (not shown) such that the "mother" processor board



(704) controls the cluster of units. The system power converter (706) supplies power to the units. Alternatively, as shown in Figure 11c, the optics circuit board (812) of each unit (810) interfaces with an individual controller board (820) so that each unit has its own controller board. In Figure 11c a thermal sleeve (814) with top support (816), optics assembly (818) and optical circuit board (812) are shown removed from the base (802).

In an array format, the gap between the units may be sealed by a gasketed plate covering the entire array. The top support and the gasketed plate may be configured to form a flush surface for a multi-unit array. The gasket material is preferably resistant to bleach and other cleaning solutions.

The base plate (802) in the array of assembly units may provide several functions. For example, the base may allow for physical mounting of the units, housing of a controller board, and electrical connection between the heat exchanging assembly units and the host computer. A multi-function electrical connector may also serve as the physical mount.

The footprint of the controlled heat exchanging unit is designed to be easily assembled into 2-dimensional arrays. In addition, the close spacing in one direction allows the use of linked linear arrays of units, if desired. In one embodiment, the overall dimensions of each assembly unit are approximately 9 x 40 x 50 mm. The narrow dimension is small enough to allow, for example, 8 units to be grouped together, if desired, in a reasonable (72 mm) length, suitable for interfacing with standard commercially available multi-pipettes which have 9 mm center-to-center spacing for convenient loading of sample and chemicals, if needed.

The thermal reaction assembly, according to the invention, may find use in many applications. The apparatus of the invention is utilized to perform chemical reactions on a sample, for example, polymerase chain reaction (PCR). The unit is provided, either directly or within a separate, insertable reaction chamber, with reagents required for the reaction. For example, in performing a polymerase chain

reaction, the chamber may include a sample polynucleotide, a polymerase such as Taq polymerase, nucleoside triphosphates, a first primer hybridizable with the sample polynucleotide, and a second primer hybridizable with a sequence complementary to the polynucleotide. Some or all of the required reagents may be present in the reaction chamber as shipped, or they may be added to the sample and then delivered through an inlet port to the reaction chamber, or the reagents may be delivered to the reaction chamber independently of the sample. The polymerase chain reaction may be performed, according to methods well known in the art.

Although polynucleotide amplification by polymerase chain reaction has been described herein, it will be appreciated by persons skilled in the art that the devices and methods of the present invention may be utilized equally effectively for a variety of other polynucleotide amplification reactions and ligand-binding assays. Such additional reactions may be thermally cycled, such as the polymerase chain reaction, or they may be carried out at a single temperature, e.g. nucleic acid sequenced-based amplification (NASBA). Moreover, such reactions may employ a wide variety of amplification reagents and enzymes, including DNA ligase, T7 RNA polymerase and/or reverse transcriptase, among others. Additionally, denaturation of polynucleotides can be accomplished by known chemical or physical methods, alone or combined with thermal change. Polynucleotide amplification reactions that may be practiced in the device of the invention include, but are not limited to: (1) target polynucleotide amplification methods such as self-sustained sequence replication (3SR) and strand-displacement amplification (SDA); (2) methods based on amplification of a signal attached to the target polynucleotide, such as "branched chain" DNA amplification; (3) methods based on amplification of probe DNA, such as ligase chain reaction (LCR) and QB replicase amplification (QBR); (4) transcription-based methods, such as ligation activated transcription (LAT) and nucleic acid sequence-based amplification (NASBA); and (5) various other amplification methods, such as repair chain reaction (RCR) and cycling probe reaction (CPR).

In addition to the aforementioned gene or target amplification methods, other chemical or biochemical reaction applications are anticipated. For example, temperature controlled lysis of cells is another application of the intended invention, which may or may not compliment gene or target amplification methods described  
5 above.. In many cases, this is accomplished by raising the temperature of the solution containing the cell to 37°C for a few minutes to allow the action of proteolytic enzymes followed by raising the temperature and holding at 95°C. After a few seconds to minutes, the cell is lysed and the target component, such as nucleic acid, is released which can then be further processe, *e.g.* amplified, without further  
10 manipulation. In other applications, it may be desired to immediately stop any further chemical reactions immediately after the lysis by lowering the temperature to 0° to 4°C, such as in the case when studying the mRNA expression state using rapid thermal polymerase chain reaction (RT-PCR). Thus the rapid thermal ramping as provided by this device enables such functionality.

15 Furthermore, the disclosed device can be utilized to control and interrogate chemical reactions. In enzyme kinetic studies, for example, it is advantageous to hold the test reaction mixture at a reduced temperature, such as 0°C - 4°C, before starting the reaction, and then to quickly bring the reaction mixture from this reduced hold temperature, *e.g.* 4°C, to an optimal reaction temperature. Unwanted side reactions  
20 occurring at intermediate temperatures and are reduced or eliminated, allowing for more accurate measurements and higher purity of product. Moreover, this approach can be extended to more complex chemical and biochemical reactions that can be controlled and studied by enabling changes to multiple different temperatures, or to periodically reduce the temperature to stop the reactions.

25 Such temperature control can be exploited for ligand binding reactions such as fluorescence homogenous immunoassays. Because the reaction start event can be precisely executed and the subsequent reaction hold temperature accurately controlled without thermal gradients, better assay performance may be achieved.

Other applications of the invention are intended to be within the scope of the invention where those applications require the transfer of thermal energy to a chemical reaction.

5 The present invention has been described above in varied detail by reference to the particular embodiments and figures. However, it is to be further understood that other modifications or substitutions may be made to the devices and methods described based upon this disclosure without departing from the broad scope of the invention.

## 5 WHAT IS CLAIMED IS:

1. A chemical reaction chamber having improved heat exchanging properties, comprising:

- a) a reservoir for receiving and holding a sample;
- b) a sealable port for introducing the sample into the reservoir,;
- 10 c) a support housing; and
- d) a thin conformable material defining a portion of at least one wall of the reservoir and attached to the support housing, said material conformable to the surface of a heat transfer element, thereby providing improved heat exchange with the sample.

15 2. The chamber of claim 1 wherein said flexible material comprises a film.

3. The chamber of claim 2 wherein the thickness of the film is about .0002 inch to .01 inch.

4. The chamber of claim 2 wherein the film comprises a polymer or metal.

5. The chamber of claim 4 wherein the film comprises a polymer selected from  
20 the group consisting of polyacrylics, polyamides, polycarbonates, polyesters, polyolefins and blends thereof.

6. The chamber of claim 2 wherein the film comprises a laminate.

7. The chamber of claim 6 wherein the laminate comprises a polymer/metal film.

8. The chamber of claim 2, wherein the film is adapted to transmit radiation.

25 9. The chamber of claim 1, further including a seal for closing said port.

5 10. The chamber of claim 9, wherein said seal is a plug insertable into the port thereby increasing pressure within said chamber.

11. A chemical reaction chamber comprising:

two opposing major faces;

a plurality of contiguous minor faces joining said major faces to each  
10 other to form an enclosed chamber wherein two minor faces are offset at an angle and are adapted to transmit radiation;

a port for introducing a sample into said chamber; and

a seal for closing said chamber;

in which the ratio of the thermal conductance of the major faces to that of the  
15 minor faces is at least about 2:1.

12. A chamber of claim 11, wherein the seal is a plug insertable into the port thereby creating increased pressure within said chamber.

13. The chamber of claim 12 in which the seal has a flexible arm attached to said chamber.

20 14. The chamber of claim 11 in which said faces are fabricated from polyacrylics, polyamides, polycarbonates, polyesters, polyolefins, and, blends of polymers.

15. The chamber of claim 11 in which the ratio of the total surface area of the major faces to that of the minor faces is at least about 2:1.

16. The chamber of claim 11 in which the angled minor faces are offset about 90°  
25 from each other.

- 5 17. The chamber of claim 11 in which a plurality of light transmissive elements are associated with the angled minor faces.
18. The chamber of claim 17 having at least one lens on each of the angled minor faces.
19. The chamber of claim 11, wherein at least one of said major faces is adapted  
10 for optical interrogation of said chamber.
20. The chamber of claim 11 wherein the angled minor faces are fabricated from a different material than that of the remaining faces.
21. The chamber of claim 11 wherein the chamber volume is from about 1 microliter to about 500 microliters.
- 15 22. The chamber of claim 11 further comprising an insert inside said chamber having a channel therein.
23. A chemical reaction chamber comprising:
- two opposing major faces in which at least a portion is a film;
- a plurality of contiguous minor faces joining said major faces to each  
20 other to form an enclosed chamber having an angled bottom;
- a port for introducing a sample into said chamber; and
- a seal for closing said chamber,
- in which the ratio of the surface area of the major faces to that of the minor faces is at least about 2:1, and the minor faces forming the angled bottom are adapted  
25 to transmit light.

- 5 24. The chamber of claim 23, wherein the seal is a plug insertable into the port thereby creating increased pressure within said chamber.
25. The chamber of claim 23 wherein the film material is selected from the group consisting of polyacrylics, polyamides, polycarbonates, polyesters, polyolefins and blends thereof.
- 10 26. The chamber of claim 23 wherein the minor faces forming the angled bottom are offset about 90° from each other.
27. The chamber of claim 23 wherein a plurality of light transmissive elements are present on the minor faces forming the angled bottom.
28. The chamber of claim 23 wherein the minor faces forming the angled bottom  
15 are fabricated from a different material than that of the remaining faces.
29. The chamber of claim 18 wherein the chamber volume is from about 1 microliter to about 500 microliters.
30. A method of making a chemical reaction chamber, comprising the steps of:
- molding a housing having a face which defines at least a portion of a  
20 reservoir and a port for introducing a sample into said reservoir;
- covering said reservoir with a thin conformable film; and
- adhering the film onto at least a portion of the face.
31. The method of claim 30, wherein the adhering the film is by welding, brazing or binding adhesives.
- 25 32. A method of thermally reacting chemicals comprising the steps of:



5           applying a sample to a reaction chamber comprising a port, a support housing, a reservoir which is adapted to receive the sample and a thin material defining at least a portion of the reservoir;

          causing the conformable material of the chamber to press against an external heat exchanging element, thereby allowing said structure to conform to  
10       the external heater surface;

          activating the external heater thereby heating said sample; and

          reacting said sample with chemicals in the chamber reservoir.

32.     The method of thermal reacting chemicals of claim 31, wherein said causing the flexible structure to press against the external heater is by outwardly expanding  
15       said structure.

33.     The method of thermal reacting chemicals of claim 31, wherein said causing the flexible structure to press against the external heater is by inwardly biasing said external heater against said flexible structure.

34.     The method of thermal reacting chemicals of claim 31, further including  
20       optically viewing the reaction.

35.     A thermal chemical reaction sleeve comprising:

          a slot for introducing a chemical reaction chamber of claim 11 into said sleeve;

          a contact surface on at least one wall of said sleeve abutting a portion of  
25       the chamber;

          a heat exchanging element integrated with at least one wall of said sleeve; and

5                   an electrical connection for applying current to said heat exchanging  
element.

36.    The sleeve of claim 35, wherein the geometry of the contact surface matches  
that of the chamber.

37.    The sleeve of claim 35 further including a spring for biasing the contact surface  
10   with a portion of the sample sleeve.

38.    In a thermal chemical reaction sleeve, the improvement comprising:

          a contact surface defining at least a portion of said sleeve and having integrated  
heating elements; and

          a slot for introducing a sample vessel into said sleeve.

15   39.    The sleeve of claim 38, further including a biasing element for applying  
pressure on said contacting surface to press against the sample vessel positioned in the  
sleeve.

39.    The sleeve of claim 38 wherein the material of fabrication is selected from the  
group consisting of ceramics, metals, and synthetic polymers.

20   40.    The sleeve of claim 38 wherein the ceramics are selected from the group  
consisting of aluminum nitride and aluminum oxide.

41.    The sleeve of claim 38 wherein the sleeve volume is from about 10 $\mu$ l to about  
10 ml.

42.    The sleeve of claim 38 wherein the integrated heating elements are thick or thin  
25   film resistors.

43.    The sleeve of claim 42 wherein the size and shape of the film resistors varies  
with their location in the sleeve wall.

- 5 44. The sleeve of claim 38 further comprising optically transmissive elements on two walls thereof.
45. In a thermal chemical reaction sleeve, the improvement comprising:
- two opposing plates having integrated heating elements;
  - a slot for introducing a sample vessel between said plates;
  - 10 a support plate for holding said opposing plates; and
  - a biasing element for applying pressure on said opposing plates, whereby the opposing plates press against a sample vessel there between.
46. The sleeve of claim 45 wherein the material of fabrication is selected from the group consisting of ceramics, metals, and synthetic polymers.
- 15 47. The sleeve of claim 45 wherein the ceramics are selected from the group consisting of aluminum nitride and aluminum oxide.
48. The sleeve of claim 45 wherein the sleeve volume is from about 2 $\mu$ l to about 10ml.
49. The sleeve of claim 45 wherein the heating elements are thick or thin film  
20 resistors.
50. The sleeve of claim 49 wherein the size and shape of the film resistors varies with their location in the sleeve wall.
51. The sleeve of claim 49 further comprising an optically transmissive element on a wall thereof.
- 25 52. A heat exchanging chemical reaction module comprising:

5 a thermal sleeve comprising:

a body having heating elements integrated with at least one of the walls of the body; and

a top support having a slot for introducing a sample chamber into said sleeve; and

10 a sample chamber at least partially inserted between the walls of said sleeve comprising:

a sealable port for introducing the sample into said reservoir;

a support;

a reservoir which is adapted to receive the sample; and

15 a thin flexible material defining at least a portion of the reservoir and coupled to said rigid support, said flexible material conformable to the surface of the sleeve heating wall,

wherein said flexible structure of the sample chamber contacts the sleeve heating wall for optimal thermal conduction.

20 53. The module of claim 52 further including an optics assembly in optical communication with said sample chamber.

54. A controlled heat exchanging instrument comprising:

a housing unit comprising:

an electrical connection;

25 a cooling source;

- 5 a heating element;
- a processing area adapted to receive a reaction vessel for holding a sample; and
- an optics assembly in optical communication with said processing area;
- a circuit board for controlling optics assembly and for collecting the
- 10 output signal of the optics system; and
- a microprocessor for independently controlling the operation of the unit.

55. The instrument of claim 54 wherein the optics assembly comprises light transmissive windows integrated into the thermal sleeve, lensing elements in optical communication with said windows, a light emitting source and light detector.

15 56. The instrument of claim 54 further comprising a heating and cooling indicator LED.

57. A controlled heat exchanging system comprising:

- a base plate, said base plate having electrical connection;
- at least one housing sealably mounted on said base plate, in electrical
- 20 connection therewith, said housing having internal support and cooling system;
- a module inserted into said housing, said module comprising an optics assembly, a thermal sleeve having an integrated heating element, and a slotted top plate; and
- circuit for controlling the optics assembly and for collecting the output
- 25 signal of the optics assembly.

- 5 58. The system of claim 57 wherein the optics assembly comprises light transmissive windows integrated into the thermal sleeve, lensing elements in optical communication with said windows, a light emitting source and light detector.
59. The system of claim 57 further comprising a heating and cooling indicator LED.
- 10 60. The system of claim 57 wherein each thermal sleeve has integrated heating elements.
61. A method for performing a heat exchanging chemical reaction comprising inserting the sample to be reacted into said processing area of an instrument of claim 54 and initiating the desired operation.
- 15 62. A method for performing a heat exchanging chemical reaction comprising inserting the sample to be processed into the reaction module of an assembly of claim 57 and initiating the desired operation.
63. A controlled heat exchanging system comprising:
- a base plate, said base plate having an electrical connection and a
- 20 controller board;
- a plurality of controlled heat exchanging assemblies, each of said assemblies comprising:
- a housing coupled to said base plate, in electrical connection therewith, said housing having a cooling system, and an integrated circuit board or chip; and
- 25 a module inserted into said housing, said module comprising an optics assembly, a thermal sleeve having integrated heating elements, and a slotted top plate,

5            wherein the optics assembly comprises light transmissive windows integrated into the thermal sleeve, lensing elements in optical communication with said windows, a light emitting source and light detecting sensor; and

             wherein said controller board independently monitors and controls each assembly and collects and analyzes the output signal of each assembly.

10    64.    A controlled heat exchanging process comprising inserting the sample to be processed into a reaction module of a system comprising:

             a base plate, said base plate having an electrical connection and controller board, said base plate having a plurality of controlled heat exchanging assemblies in electrical connection with said base plate, each of said assemblies  
15    comprising:

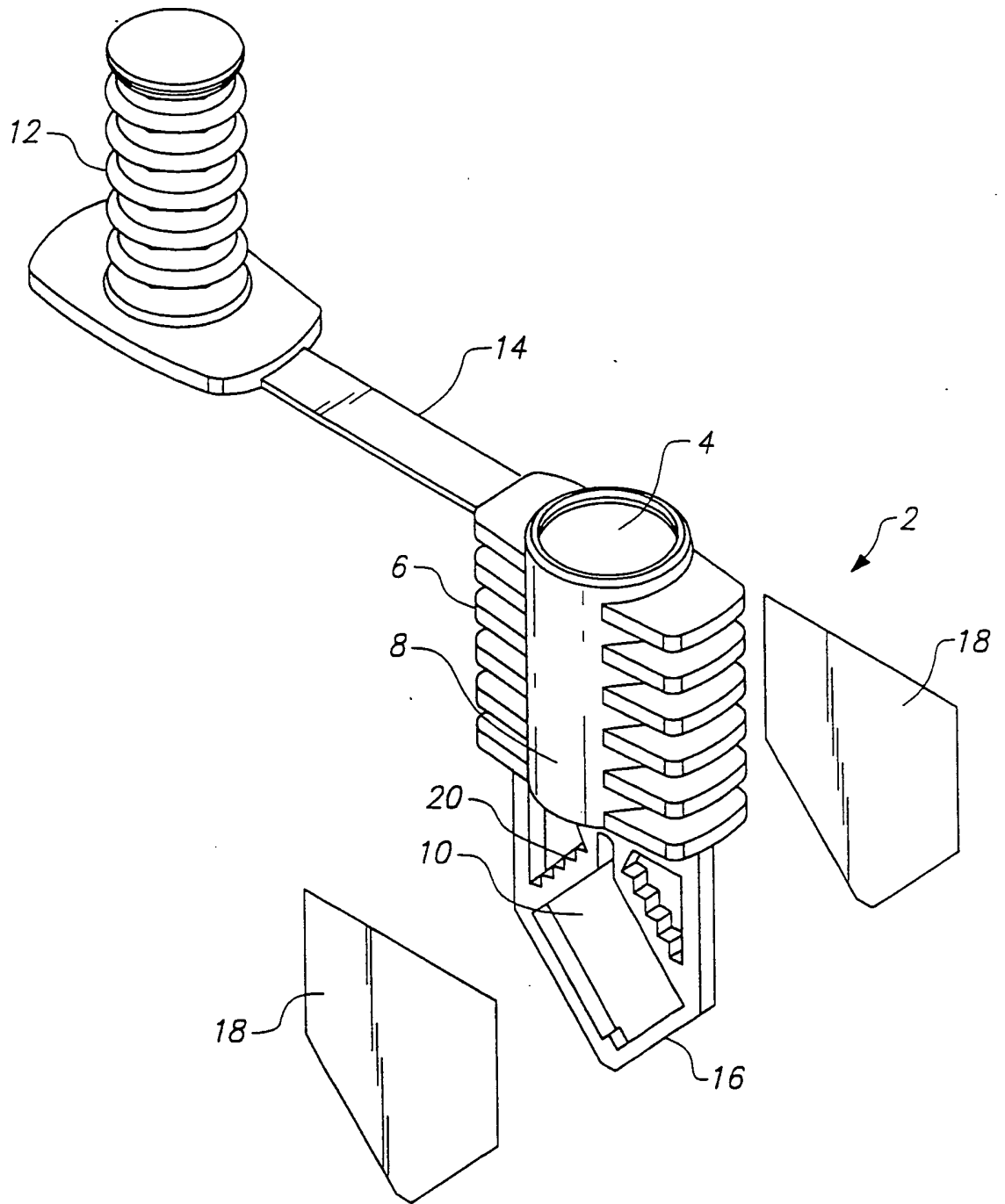
             a housing including a cooling system, and an integrated circuit board or chip;

             a module inserted into said housings, said module comprising an optics assembly, a thermal sleeve having an integrated heating element, and a slotted top  
20    plate;

             wherein the optics assembly comprises light transmissive windows integrated into the thermal sleeve, lensing elements in optical communication with said windows, and light emitting and detecting sensors; and

             wherein said controller board independently monitors and controls each  
25    assembly and collects and analyzes the output signal of each assembly; and initiates the desired operation.

65.    The process of claim 64 wherein different heat exchanging chemical reactions are performed simultaneously in separately controlled assemblies.



**FIG. 1**



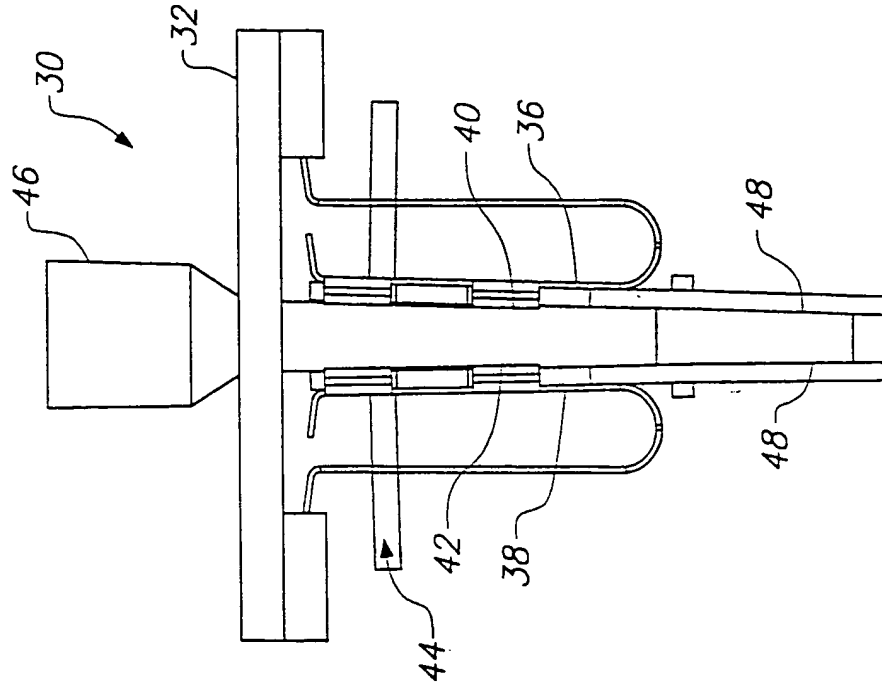


FIG. 2a

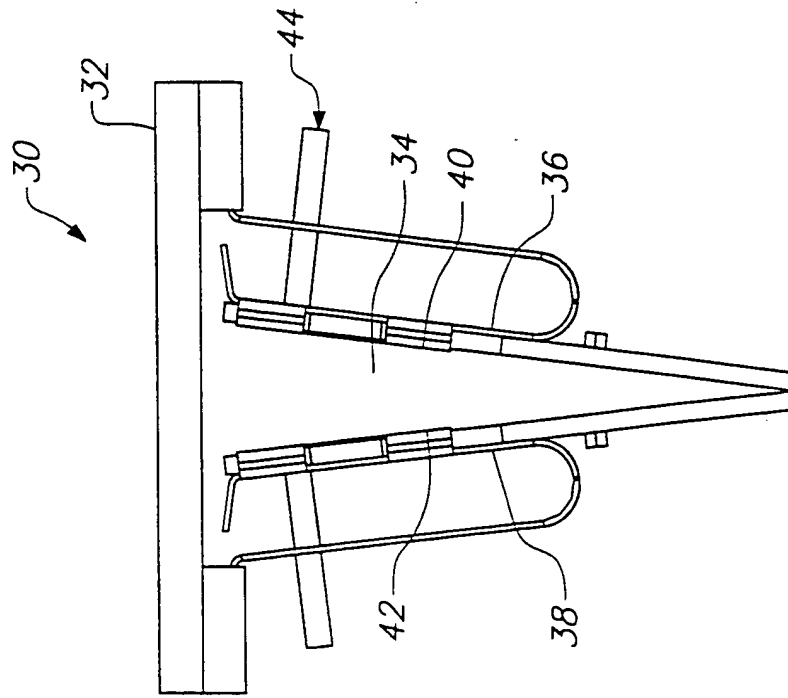
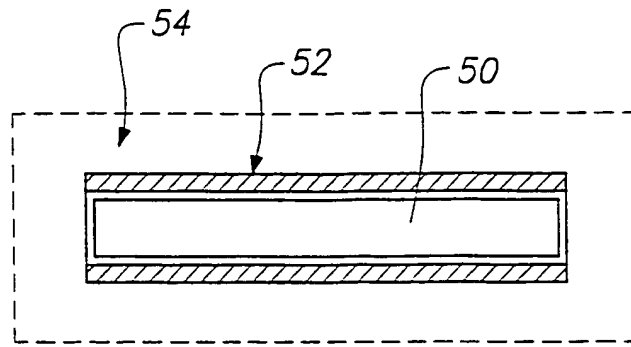
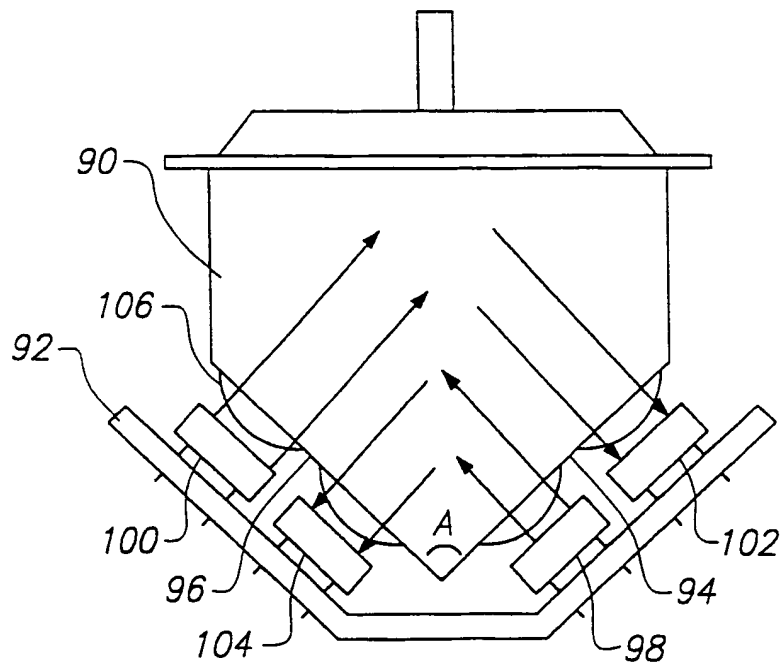


FIG. 2b



**FIG. 3**



**FIG. 5**

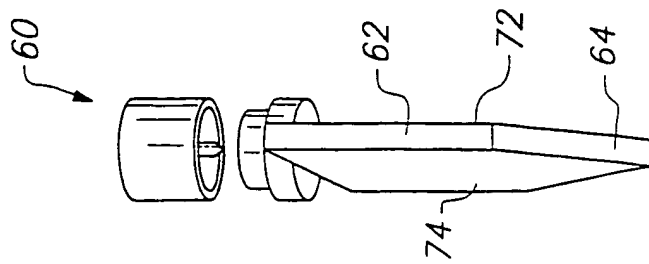


FIG. 4a

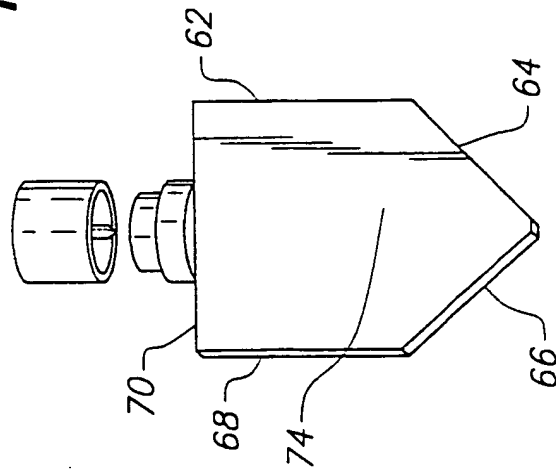


FIG. 4b

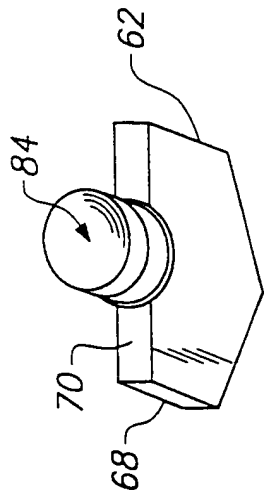


FIG. 4d

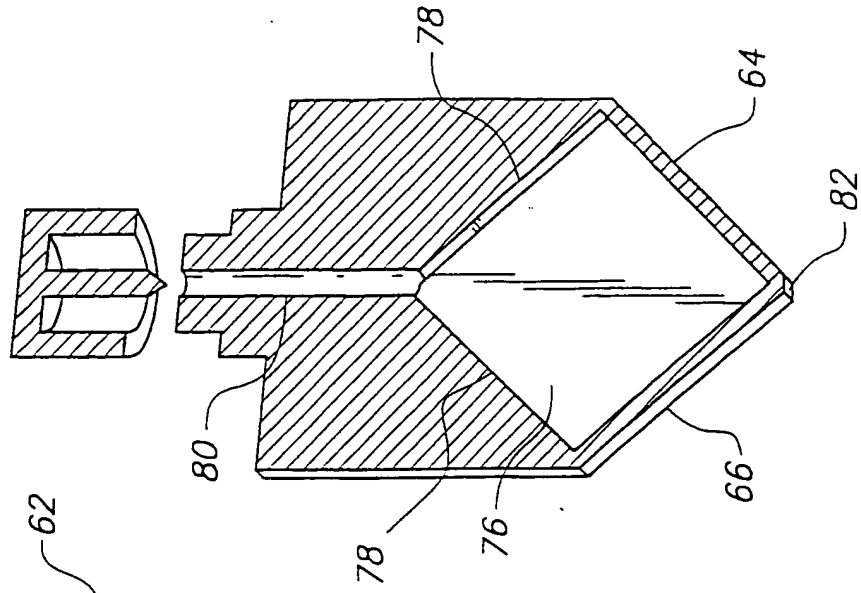
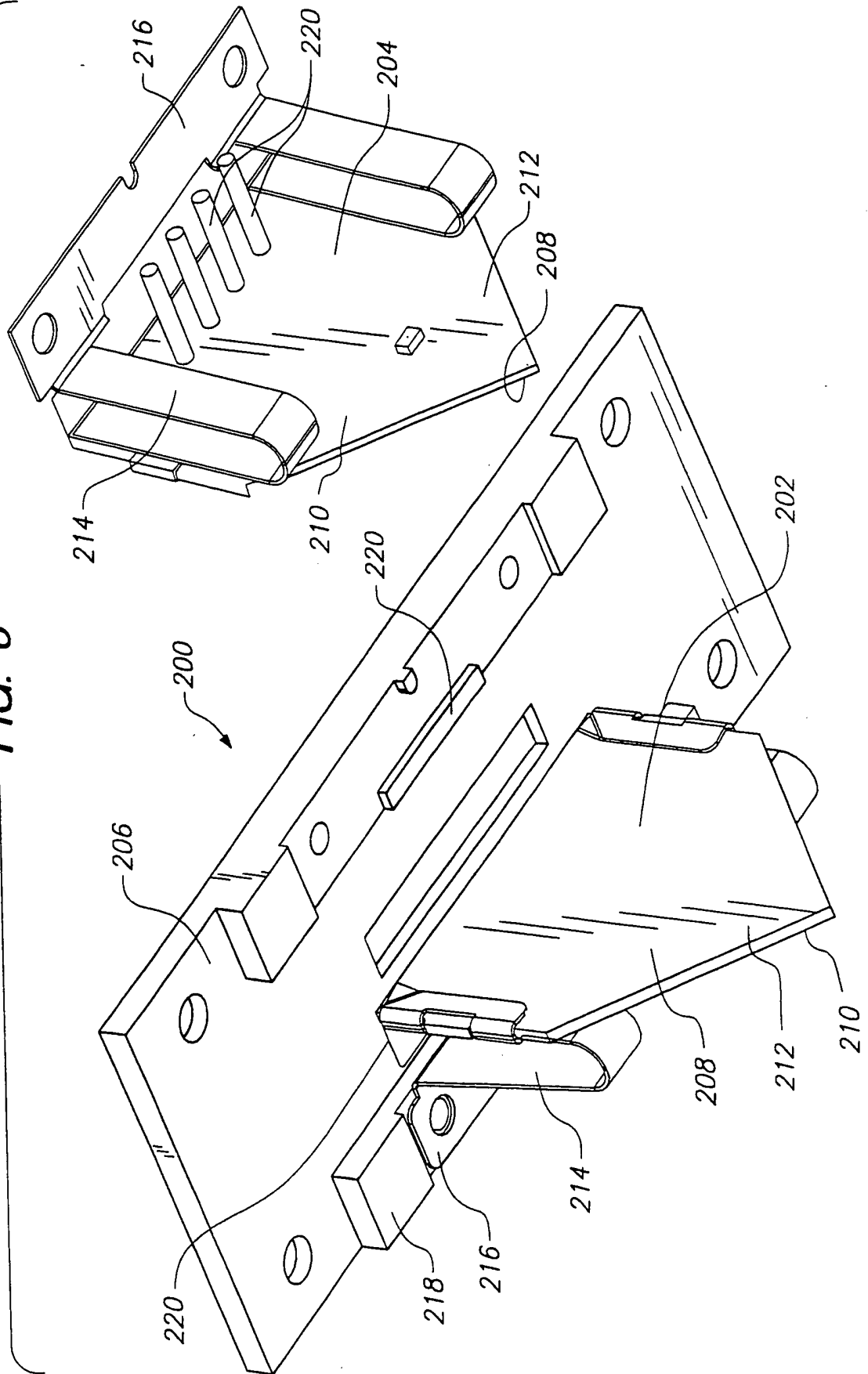


FIG. 4c

FIG. 6



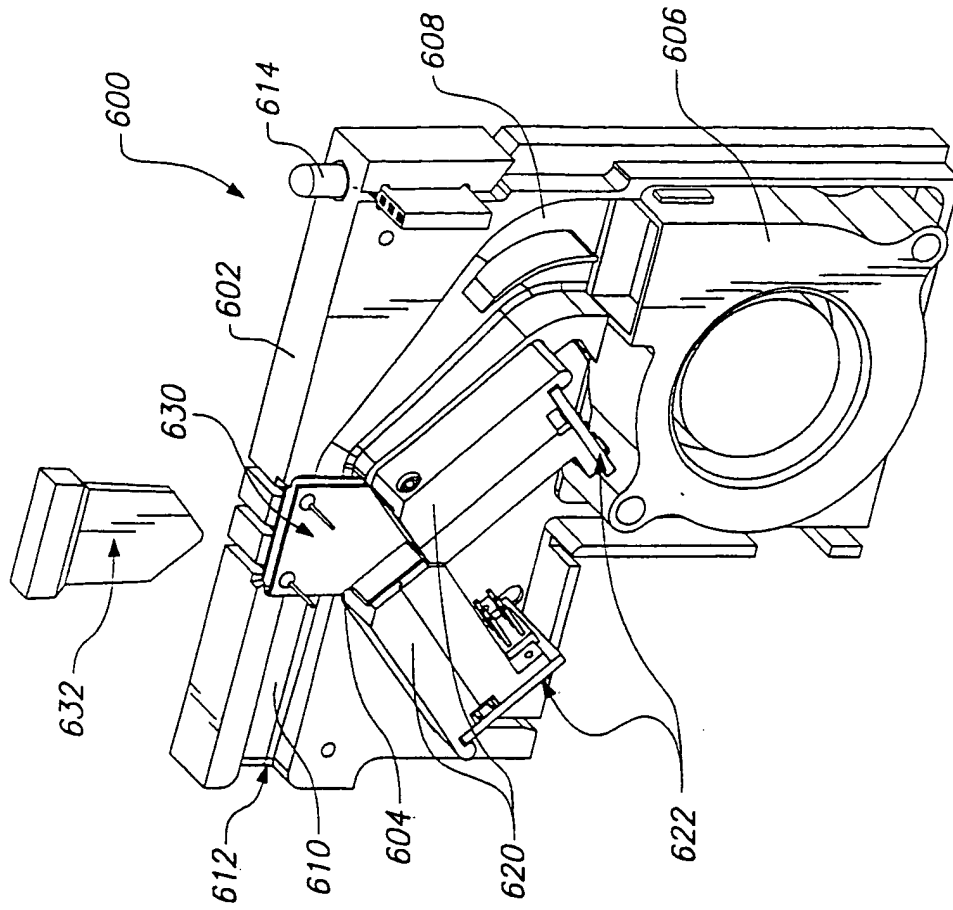


FIG. 9

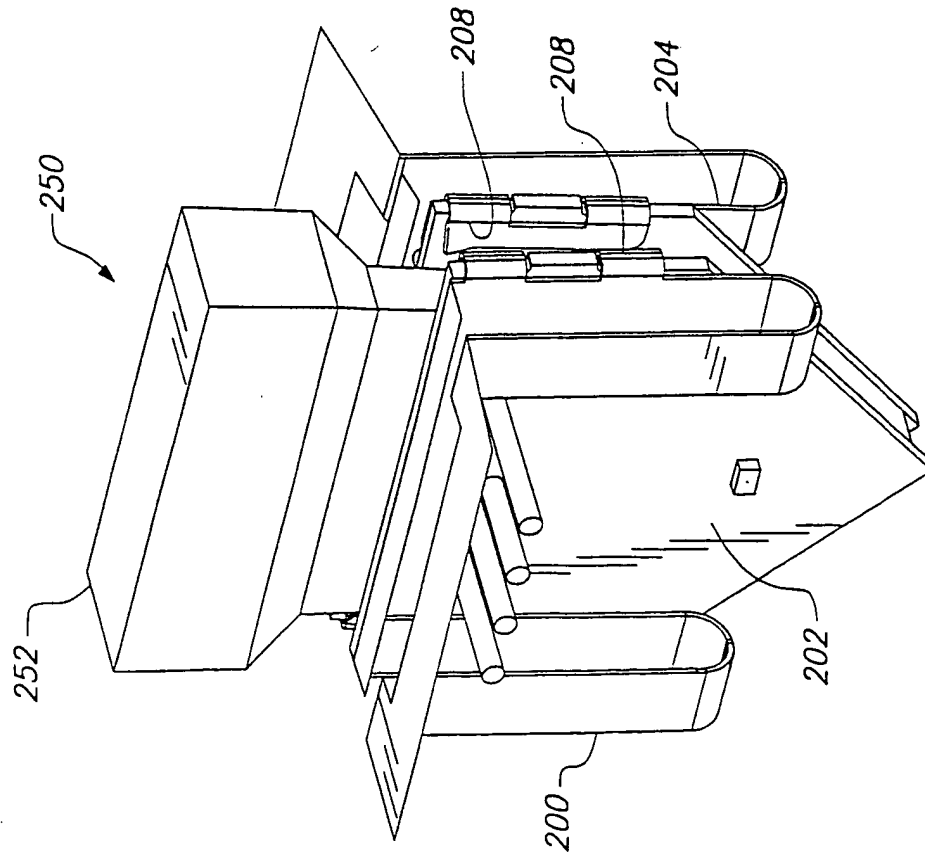


FIG. 7

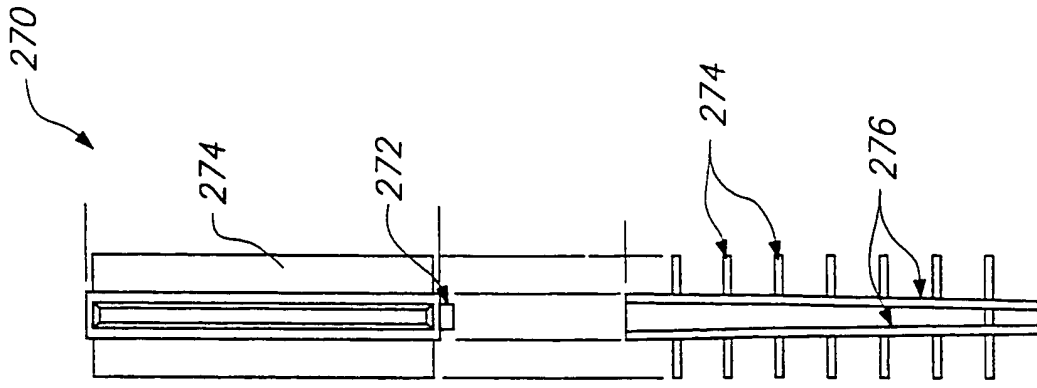


FIG. 8d

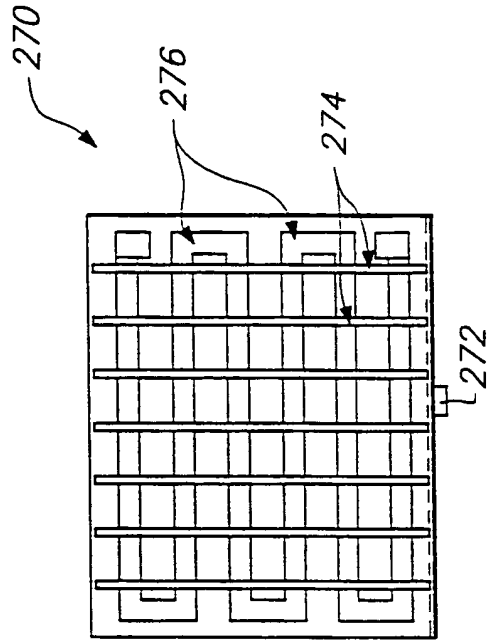


FIG. 8c

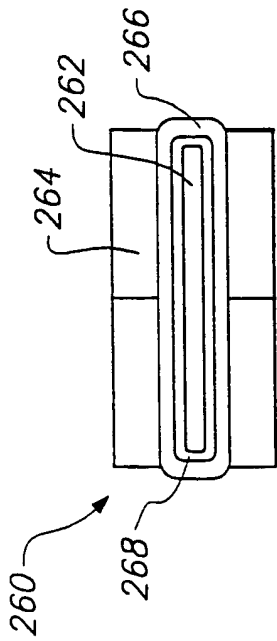


FIG. 8a

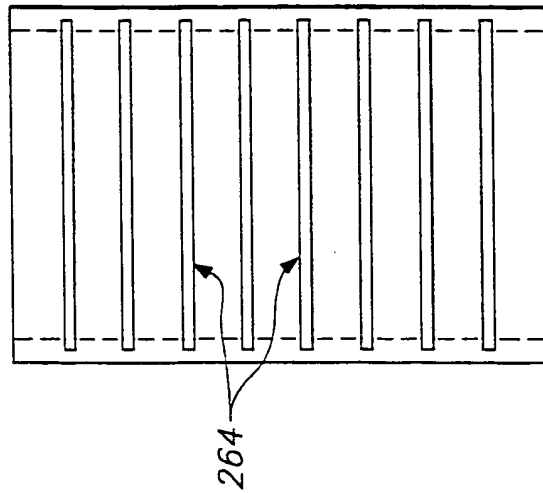


FIG. 8b

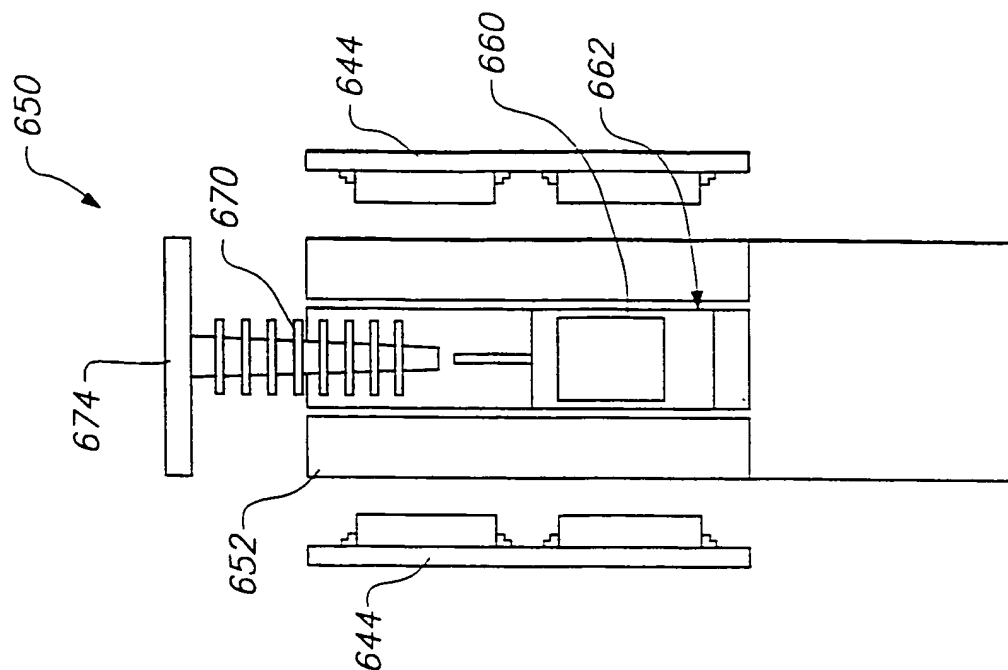


FIG. 10b

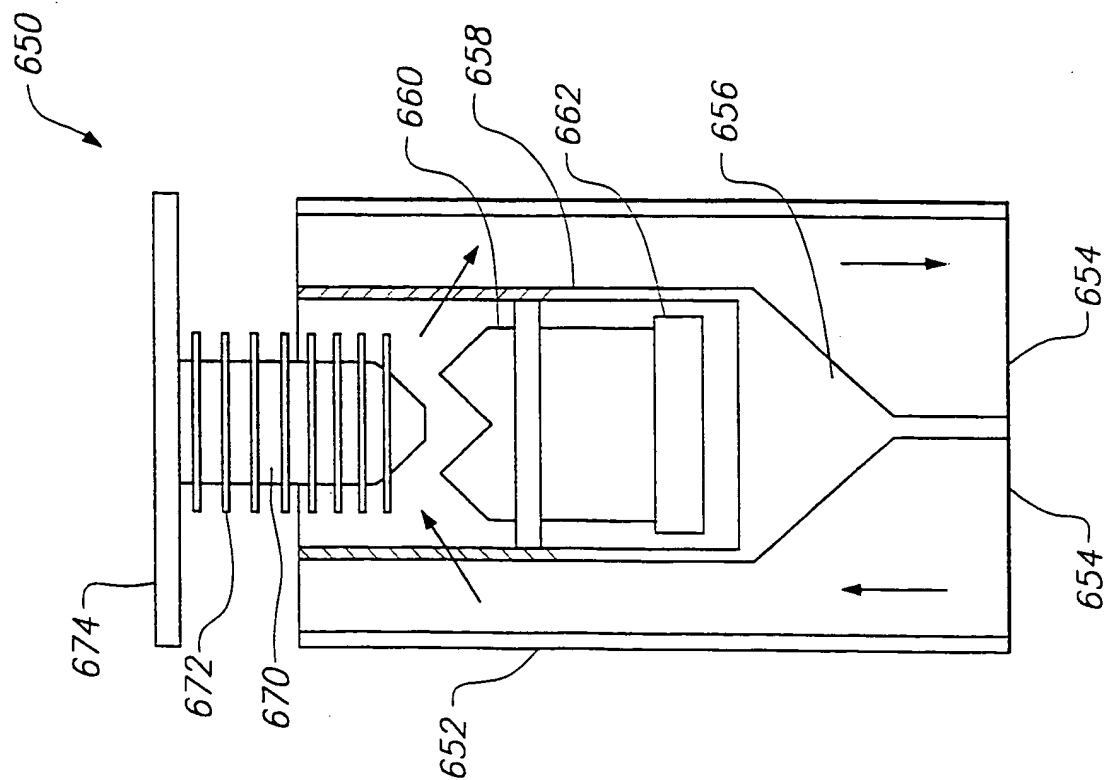
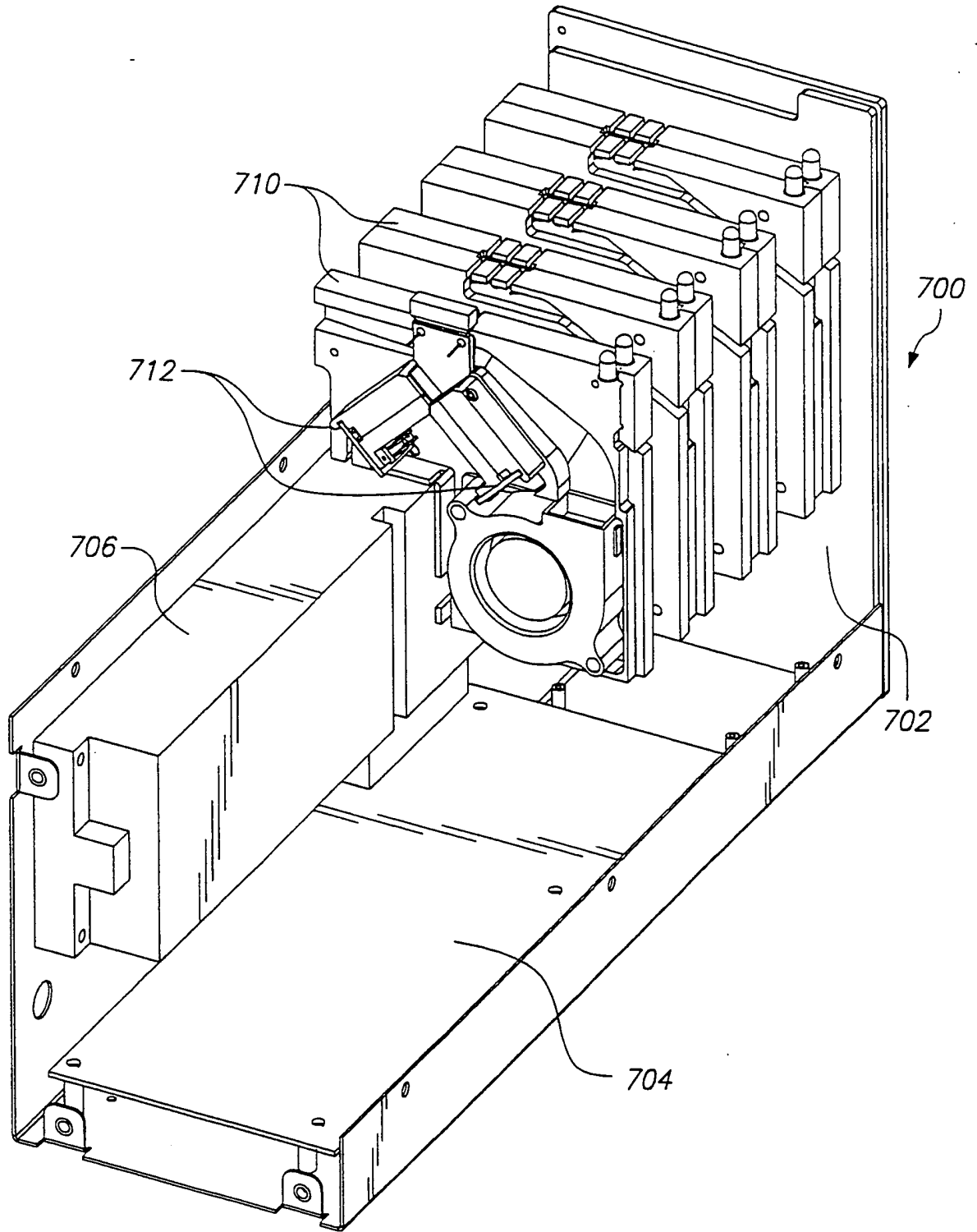


FIG. 10a

**FIG. 11a**



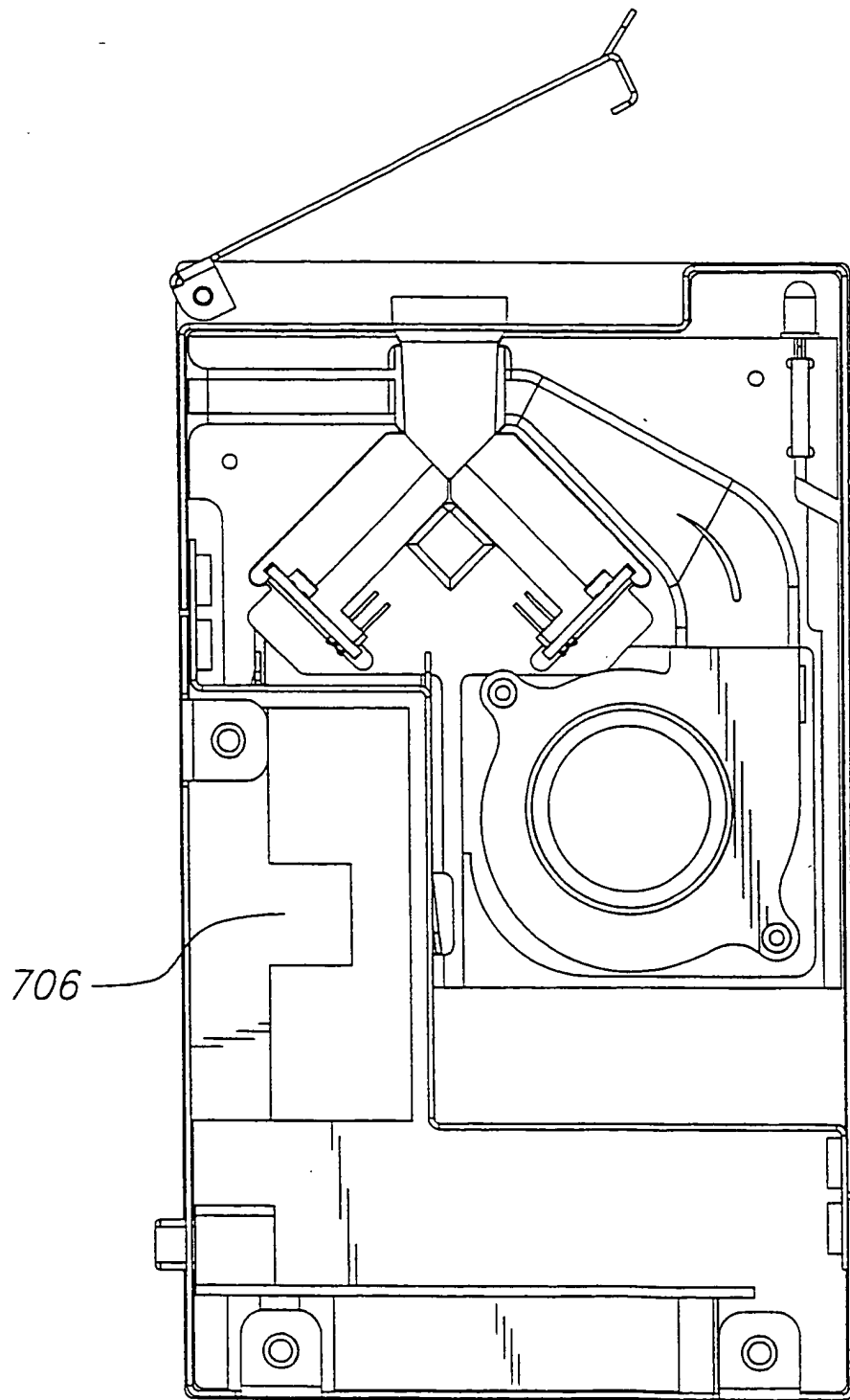
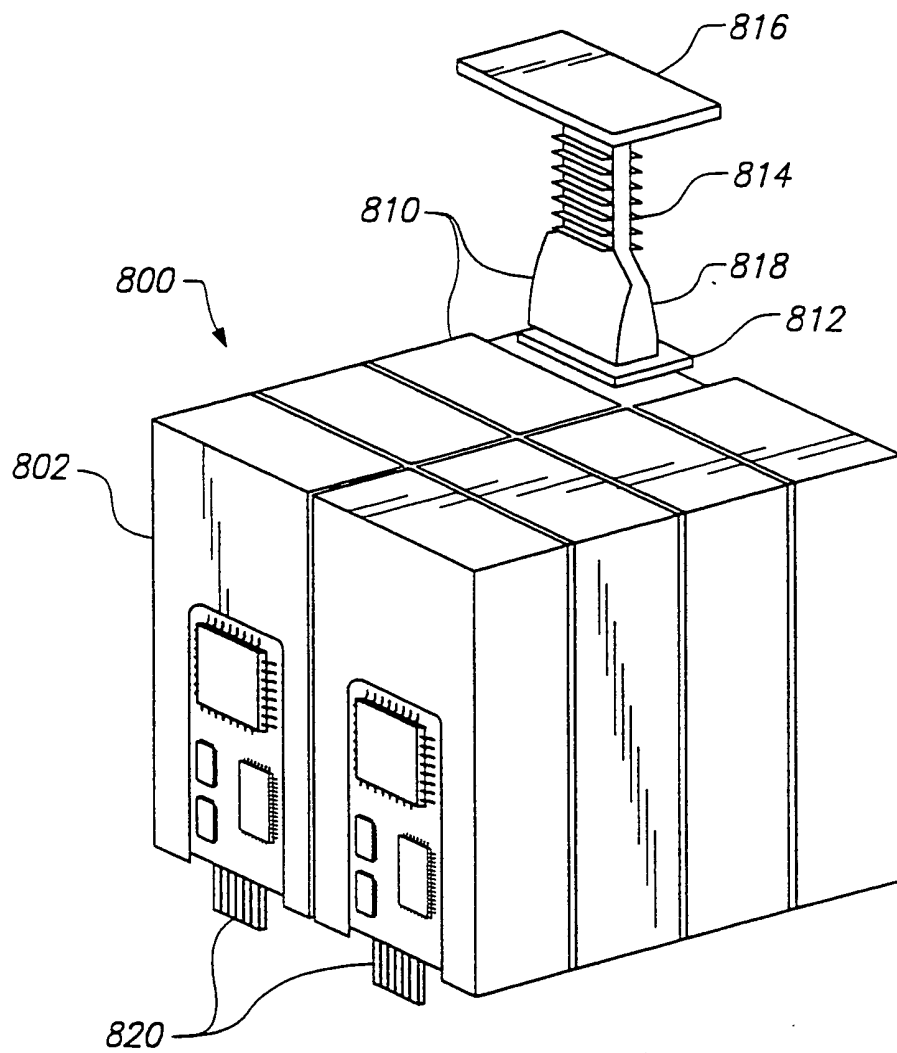


FIG. 11b



**FIG. 11c**

## A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 C1201/68 B01L7/00 G01N21/03

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 B01L G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category | Citation of document, with indication, where appropriate, of the relevant passages  | Relevant to claim No. |
|----------|---|-----------------------|
| A        | EP 0 606 961 A (EASTMAN KODAK CO) 20 July 1994  | 30                    |
| X        | see column 3, line 45 - column 4, line 16<br>see column 7, line 7 - column 8, line 11<br>---  | 1                     |
| X        | EP 0 662 345 A (CLINICAL DIAGNOSTIC SYST)<br>12 July 1995<br>see column 5, line 35 - column 6, line 25;<br>figure 6<br>see column 8, line 2 - line 52; figures<br>7-10<br>see column 9, line 1 - line 21 .<br>--- | 1-9,<br>32-34         |
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Further documents are listed in the continuation of box C.



Patent family members are listed in annex.

## \* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&amp;" document member of the same patent family

Date of the actual completion of the international search

21 August 1998

Date of mailing of the international search report

09.09.98

Name and mailing address of the ISA

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Fax: (+31-70) 340-3016

Authorized officer

Hocquet, A

| C. (Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT |   |                       |
|---|---|-----------------------|
| Category  | Citation of document, with indication, where appropriate, of the relevant passages  | Relevant to claim No. |
| X   | US 5 589 136 A (NORTHROP M ALLEN ET AL)<br>31 December 1996<br>cited in the application<br>see column 7, line 36 - column 8, line 12;<br>figures 4,6,12-14<br>see column 12, line 45 - column 13, line 18;<br>figures 12-14 | 38-51                 |
| X   | see column 12, line 5 - line 16; figures 4,10   | 52-65                 |
| X   | see column 13, line 19 - column 14, line 7; figures 15-17   | 52-65                 |
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| A   | EP 0 603 411 A (KATOHI KEIICHI ;UEDA SYUZI (JP)) 29 June 1994<br>see claims 1,18; figures   | 39,40                 |
| A   | EP 0 545 736 A (BELL JOHN IRVING ;OLD JOHN MICHAEL (GB); POTTER COLIN GERALD (GB);) 9 June 1993<br>see page 5, line 1 - line 18   | 43                    |
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## INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 98/ 03962

**Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)**

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:  
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

**Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)**

This International Searching Authority found multiple inventions in this international application, as follows:

1. CLAIMS 1-10, 30-31, 32-34, 38-51, 52-53
2. CLAIMS 11-22, 23-29, 35-37, 54-65

FOR FURTHER INFORMATION PLEASE SEE FORM PCT/ISA/206 MAILED 22.06.98

1. ☒ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest
- ☒ No protest accompanied the payment of additional search fees

## FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1-10,30-31,32-34,38-51,52-53

chemical reaction chamber with thin conformable portion  
improving heat exchange

2. Claims: 11-22,23-29,35-37,54-65

chemical reaction chamber with minor faces offset at an  
angle and adapted to transmit radiation.

| Patent document<br>cited in search report | Publication<br>date | Patent family<br>member(s) | Publication<br>date |
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